University of Alberta

The Genetics of Earliness in Canadian Spring Wheat (*Triticum aestivum* L.)

by

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Canada

To

Mom and Dad

And

Nargis, Hassan, Tayyab and Sadaf

Abstract

Early maturing spring wheat (*Triticum aestivum* L.) cultivars are required for the short growing season of western Canada. However, earliness in wheat is an extremely complex trait controlled by photoperiod response, vernalization and earliness *per se* genes. The objectives of this thesis were to investigate the genetic control and inheritance of flowering and maturity in Canadian spring wheat, the effects of vernalization response *(Vrn)* genes on important agronomic traits and the association of days to maturity with grain yield and protein. Five spring wheat cultivars covering the range of maturity of western Canadian spring wheat were first studied under 10 and 16 hour photoperiods, and 0 and 42 day vernalization treatments. Vernalization hastened flowering and decreased final leaf number in 'AC Foremost' and 'AC Taber'. Thereafter, the five parents and their F_1 hybrids obtained from a one-way diallel cross were grown with and without vernalization treatment in a growth chamber. These genotypes, and the F_2 generation of the diallel cross, were also tested in the field for two years. Flowering and maturity times were altered by both *Vrn* and earliness *per se* genes that mainly acted additively. High yielding and late Canadian Prairie Spring wheat cultivars 'AC Foremost' and 'AC Taber' were found to have different spring habit *Vrn* alleles than 'AC Barrie', 'AC Intrepid' and 'Cutler'. Molecular characterization of 42 Canadian spring wheat genotypes uncovered the presence of spring habit alleles of *Vrn-A1* and *Vrn-B1* in 83% and 50% of the genotypes, respectively. Thirty six percent of the genotypes had both of these alleles in combination. All genotypes carried the winter habit allele of *Vrn-D1*. A four environment field evaluation of 16 genotypes of known *Vrn* genes revealed that genotype with three spring habit alleles at the major *Vrn* (*Vrn-A1 Vrn-B1 Vrn-D5*) loci matured early but had

low grain yield. Genotypes with spring habit alleles *Vrn-A1* and *Vrn-B1* were early and relatively high yielding. Despite the positive association between maturity and grain yield, and the negative association between grain yield and protein content, higher yielding lines with medium maturity and higher grain protein were identified in a population of 130 spring wheat genotypes. Results of these studies suggest the feasibility of breeding early maturing spring wheat cultivars with relatively high grain yields in western Canada.

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List of symbols and abbreviations

bp	Base pair
CIMMYT	International wheat and maize improvement center
cm	Centimeter
d	days
eps	Earliness *per se*
GCA	General combining ability
ha	Hectare
h^2_{ns}	Narrow-sense heritability
h^2_{bs}	Broad-sense heritability
h	Hour
kg	Kilogram
m	Meter
MAS	Marker assisted selection
mg	Milligram
min	Minutes
mm	Millimeter
MP	Mid-parental value
PCR	Polymerase chain reaction
Ppd	Photoperiod
ppm	Parts per million
QTL	Quantitative trait loci
r	Coefficient of correlation
RCBD	Randomized complete block design
R_e	Expected response to selection
R_o	Observed response to selection
SCA	Specific combining ability
SE	Standard error
s	Seconds
χ^2	Chi square
t	tonne
µL	Micro-liter
µM	Micro Molar
Vrn	Vernalization

Chapter 1

Genetics of Earliness in Bread Wheat: A Review of Literature

1.1. Wheat

Bread wheat (*Triticum aestivum* L.) is the most important and widely grown food crop in the world. Wheat occupies 17% of the global crop acreage, feeds about 40% of the world's population and provides 20% of the caloric and protein requirements in human nutrition (Gupta et al., 2005). For technical purposes wheat has been divided into different classes, which include hard, medium, and soft grain texture; white, amber or red bran color; and spring or winter growth habit (Candlish, 2002).

Wheat belongs to the most common species (*aestivum*) of the genus *Triticum*. Wheat is a natural allohexaploid ($2n = 6x = 42$; AABBDD), containing three distinct but genetically related (homoeologous) genomes, A, B, and D, each with a haploid set of 7 chromosomes. Wheat originated from hybridization between the tetraploid wild emmer wheat *Triticum turgidum* ssp. *dicoccum* ($2n = 4x = 28$; AABB) and the diploid wild wheat *Aegilops tauschii* ($2n = 2x = 14$; DD). The haploid nuclear genome of *T. aestivum* contains 16.72×10^9 bp of DNA, distributed over 42 chromosome arms (Gupta et al., 2005).

Hexaploid wheat, like many other allopolyploid plant species, exhibits a diploid-like meiotic behavior to circumvent the formation of multivalent associations of more than two homologous or homoeologous chromosomes at meiosis, which can result in genetically unbalanced gametes (Naranjo & Corredor, 2004). This diploid-like chromosome pairing is genetically controlled by the *Ph1* (Pairing homoeologous) gene, located on the long arm of chromosome 5B (Riley & Chapman, 1958). The *Ph1* gene, by suppressing pairing of homoeologous chromosomes, validates the assumption of disomic inheritance in the genetic studies of hexaploid wheat.

1.2. Wheat in Canada

Wheat is the most important crop grown in Canada. Due to severe winter climates, spring wheat is commonly cultivated in Canada, with winter wheat grown on a

very small area. Most of the spring wheat in Canada is grown in the prairie provinces of Alberta, Saskatchewan, and Manitoba. Growing conditions for wheat in Canada are generally dry (350 to 550 mm precipitation annually), with a short growing season. Wheat dominates the cereal grain industry and contributes 8.3% to total farm cash receipts in Canada (Canada Grains Council, 2001). Because of its high quality and protein content, Canadian western red spring wheat has a high export demand (Curtis, 2002). Canada is the second largest exporter of wheat and wheat flour, following USA (Statistics Canada, 2001). The average annual export between 1991-92 and 2000-01 was 19.1 million tons, 18.3 % of the total world export. Normally about 80% of the total wheat produced in Canada is exported (Statistics Canada, 2001).

1.3. Early maturity

Early maturity is one of the important objectives in spring wheat (*Triticum aestivum* L.) breeding programs in Canada and elsewhere. Earliness ensures early crop harvest and may also help wheat escape from biotic and abiotic stresses such as disease, heat, drought and frost damage (Poehlman & Sleper, 1995). In western Canada, earliness is required due to the short growing season and the threat of pre-harvest sprouting of physiologically mature grains on the plant (Tames, 2005). In west central and northern areas of Alberta, wheat cultivars with early maturity, lodging tolerance and pre-harvest sprouting resistance are required (Tames, 2005). The recommended range of maturity for varieties grown in the prairie provinces is 95 to 125 days (Tames, 2005). Wheat breeders need to consider the frost free period when selecting for these regions because frost both at anthesis and later in the season can adversely affect yield and quality. In addition, early maturing cultivars need to be high yielding in order to be competitive with other crops.

Pre-harvest sprouting of grain is another reason why breeding for earliness is needed in western Canada. Physiologically mature grains of wheat can sprout before harvest if conditions are too wet (McCaig & DePauw, 1992). Pre-harvest sprouting results from the action of various hydrolytic enzymes on the starch and protein reserves of the grain (Hucl & Matus-Cadiz, 2002). As the enzymes break down the endosperm, end-use quality deteriorates. One of the factors conferring resistance to pre-harvest

sprouting is seed dormancy (Noll et al., 1982). It has been suggested that pre-harvest sprouting can be controlled to some extent by selecting for stronger seed dormancy (Hucl & Matus-Cadiz, 2002) and early maturity.

1.4. Association of early maturity with grain yield and protein content

Early maturity is negatively correlated with grain yield and protein content, posing a challenge to the development of spring wheat cultivars with early maturity, high yield and high quality (DePauw et al., 1995). Time to anthesis and the subsequent period from anthesis to physiological maturity (grain filling) are the main determinants of time to maturity (Duguid & Brule-Babel, 1994). As grain filling duration (GFD) and grain filling rate (GFR) determine final grain weight in wheat, a better understanding of the processes and factors governing maturity and grain filling may aid in decreasing time to maturity with little change in grain yield (Duguid & Brule-Babel, 1994). Existence of genetic variation for the rate and duration of the grain filling period has been reported in spring wheat (Bruckner & Frohberg, 1987; Darroch & Baker, 1990; Talbert et al., 2001). There are inconsistent reports about the associations between GFD and grain yield. Some studies have reported a positive association between GFD and grain weight and/or yield (Sofield et al., 1977; Gebeyehou et al., 1982; Sharma, 1994), while others reported no association between the two traits (Nass & Reiser, 1975; Bruckner & Frohberg, 1987; Talbert et al., 2001; Wang et al., 2002). The association between GFR and grain weight has been reported to be positive and more consistent across different experiments (Bruckner & Frohberg, 1987; Hunt et al., 1991; Darroch & Baker, 1995).

1.5. Genetic systems regulating flowering/maturity time in wheat

Due to its adaptability, wheat is cultivated from the equator to greater than 60° latitude (Briggle & Curtis, 1987). The cultivation of hexaploid wheat in a wide range of environmental conditions has resulted mainly from a directed selection of the timing of anthesis (Gororo et al., 2001). The adaptability of wheat to a particular set of environmental conditions requires adjustments in life cycle such that flowering and maturity occur at the most appropriate times (Worland, 1996). A major objective of any given wheat breeding program is to match genotypes to environments so that maximum

yield stability across locations and years can be obtained (Ortiz Ferrara et al., 1998). A thorough understanding of the genetic factors governing adaptability aids in better targeting of germplasm to specific environments, thereby reducing the risk of crop failure and ensuring maximum crop production.

Growth and developmental phases (tillering, stem elongation, ear emergence, anthesis and ripening) of wheat are controlled by vernalization (*Vrn*) and photoperiod (*Ppd*) response, and earliness *per se* genes (Kosner & Pankova, 1998). These genes, along with their interactions with growth temperatures (Gororo et al., 2001) play a significant role in the adaptation and yield potential of wheat across a diverse range of environments. Photoperiod and vernalization genes act to accelerate or delay flowering in response to seasonal changes in the environment, to ensure that flower development occurs at optimum temperatures (Law & Worland, 1997). For instance, at high northern latitudes, vernalization genes delay ear initiation in winter wheats to protect the delicate floral organs from damage due to extreme low temperatures. Similarly, in regions of the world where summers are too hot for wheat growth, photoperiod insensitive genes accelerate ear initiation and development with increasing day length during late winter and early spring to ensure the completion of the reproductive phase before the onset of high temperatures.

The *Vrn* gene system accounts for about 70-75%, the *Ppd* gene system for about 20-25% and earliness *per se* for about 5% of the genetic variability in the heading date of bread wheat (Stelmakh, 1998a). Genes influencing flowering in wheat are distributed over almost all chromosomes (Law & Worland, 1997), and genes conferring vernalization and photoperiod responses, and earliness *per se* appear to be located on each of the three homoeologous chromosomes of a group (Worland & Snape, 2001).

1.5.1 Vernalization response

Vernalization is the "acquisition or acceleration of the ability to flower by a chilling treatment" (Chouard, 1960). Wheat has both a winter and a spring growth habit. Winter wheats require exposure to a continuous cold treatment (vernalization) prior to

reproductive initiation. Spring wheats generally do not have such a requirement, but some cultivars do respond to cold by flowering earlier (Levy & Peterson, 1972; Jedel et al., 1986). The spring/winter growth habit is genetically controlled by genes that are either sensitive or insensitive to cold temperatures (Worland & Snape, 2001). The spring growth habit is manifested by the presence of one or more dominant alleles at the major vernalization loci, which confer partial or no sensitivity to cold treatment. Winter wheats, on the other hand, possess recessive alleles at all major vernalization loci, thus requiring exposure to cold temperatures for a certain time period before the onset of flowering. The winter growth habit is characterized by delayed or absence of flowering in the absence of cold temperatures following germination. Vernalization sensitivity plays a key role in adaptability, as it protects the delicate floral primordia from frost damage during winter by delaying ear initiation (Law & Worland, 1997). Although plants can respond to vernalization at any developmental stage (Flood & Halloran, 1986), the effect is more pronounced during vegetative phases (Slafer & Rawson, 1994). Vernalization can occur at any temperature between 0 and 10°C.

Major genes governing vernalization response are located on homoeologous group 5 chromosomes of hexaploid wheat. These genes include *Vrn-A1* (formerly *Vrn1*), *Vrn-B1* (formerly *Vrn2*), *Vrn-D1* (formerly *Vrn3*), and *Vrn-D5* (formerly *Vrn4*) located on chromosomes 5A, 5B, 5D and 5D, respectively (Law et al., 1976; Maystrenko, 1980; Law & Worland, 1997; Goncharov, 2003; Kato et al., 2003). Besides these major genes on group 5 chromosomes, the presence of another gene *Vrn-B4* (formerly *Vrn5*), located on the short arm of chromosome 7B has also been reported (Law & Wolfe, 1966). Kato et al. (2003) demonstrated that the former *Vrn4* gene was independent of *Vrn-D1* (both located on the long arm of chromosome 5D) and, therefore, designated it as *Vrn-D5* (a new class of *Vrn* genes).

Another series of genes conferring vernalization sensitivity have been located on homoeologous group 6 chromosomes (Islam-Faridi et al., 1996). These genes delay ear emergence with increased dosage (as opposed to *Vrn* genes on group 5 chromosomes which accelerate ear emergence with increased dosage), and accelerate it with reduced

dosage. The flowering inhibitors produced by these genes can be suppressed by promoters of the *Vrn* genes on group 5 chromosomes, resulting in accelerated ear emergence. Homoeologous group 1 chromosomes have also been reported to carry genes for vernalization sensitivity (Law & Worland, 1997). The presence of vernalization genes on chromosome 3B has been reported (Miura & Worland, 1994). Group 4 chromosomes have been found to carry orthologous *Vrn-2* series of genes for vernalization sensitivity, with *Vrn-A2* located on a segment of chromosome 4A (Dubcovsky et al., 1998).

Among the known vernalization response genes, *Vrn-A1* has the strongest effect on inhibiting the vernalization requirement, followed by *Vrn-D1*, *Vrn-D5* and *Vrn-B1*, respectively (Goncharov, 2004). This implies that, other genetic factors remaining constant, plants with dominant *Vrn-A1* will head first, whereas those having dominant *Vrn-B1* will head last. Shindo et al. (2003), however, reported that the strong spring habit of *Vrn-B1* is equivalent to *Vrn-A1*. Existence of multiple alleles for all of the dominant *Vrn* genes has been reported (Klaimi & Qualset, 1974; Law et al., 1976; Kuspira et al., 1986). Besides being the strongest inhibitor of vernalization requirement, *Vrn-A1* is also epistatic to the dominant *Vrn-B1*, *Vrn-D1*, and *Vrn-D5* genes (Pugsley, 1971; 1972). Roberts and Larson (1985), however, reported that *Vrn-A1* is neither always fully dominant nor always epistatic. Stelmakh (1993) studied the effects of *Vrn* genes on heading time and other agronomic traits in three sets of isogenic lines of bread wheat, and reported that *Vrn* genes act as non-complementary genes with classical (*Vrn-A1*) or incomplete (*Vrn-D1*) dominant epistasis.

Berry et al. (1980) studied the effect of vernalization on near-isogenic lines of wheat differing only in *Vrn* gene(s). They observed two types of gene action for vernalization response in these lines. Lines possessing *Vrn-A1 Vrn-B1* and/or *Vrn-A1 vrn-B1* genotypes exhibited a "threshold" response to vernalization. Such lines did not respond to a cold treatment of up to 4 weeks, but did respond to cold treatment of 5 weeks or greater. Another response was cumulative, increasing with an increase in vernalization duration up to 7 weeks, after which no significant response was observed.

This response type involved *vrn-A1 Vrn-B1* and *vrn-A1 vrn-B1* genotypes. In both types *vrn-B1* intensified the response but did not alter any of them.

Vernalization affects floral initiation time, leaf number, timing of other growth stages up to emergence of the flag leaf, and tiller number (Levy & Peterson, 1972). Final leaf number has been used most frequently by researchers to indicate vernalization response. According to Chouard (1960), vernalization response cannot be proven unless there is a decrease in the final leaf number of sensitive genotypes. Several studies have confirmed a reduction in final leaf number in sensitive genotypes following vernalization (Cooper, 1956; Wang et al., 1995a, b; Brooking, 1996). Once the vernalization requirement is saturated, there is no further decrease in leaf number (Cooper, 1956). This may be due to the fact that when vernalization sensitive plants are exposed to temperatures in the vernalization range (0-10°C), their vegetative phase is shortened and the reproductive phase is accelerated, resulting in reduced leaf number compared to unvernalized plants (Wang et al., 1995a)

While studying the geographical distribution of *Vrn* genes in wheat, Goncharov (1998) found the frequencies of *Vrn-D1* to be very high in countries near the equator and in Central Asia. Landraces from Pakistan, Afghanistan and China also had this gene. Dominant *Vrn-D5* was found in one landrace originating from China. Spring habit in European wheat varieties appeared to be controlled mainly by the dominant *Vrn-B1*, and the gene pool generally employed by European wheat breeders lack both *Vrn-D1* and *Vrn-D5* genes. Iwaki and Kato (1998) crossed spring landraces from various parts of the world with a series of near-isogenic lines of 'Triple Dirk', each with a specific pair of vernalization genes (*Vrn-A1*, *Vrn-B1*, *Vrn-D1* or *Vrn-D5*). Genetic analysis of the F_2 population suggested that most landraces possessed *Vrn-A1* and *Vrn-D1* followed by *Vrn-B1*. Only 8 landraces possessed the single dominant *Vrn-D5* gene. They noted that 46% of landraces carrying *Vrn-A1* belonged to areas with average January temperature below -7°C while 60% of *Vrn-A1* carrier landraces were being grown in areas above 10°C, suggesting that *Vrn-A1* confers adaptability to warmer environments. A recent study concluded that *Vrn-D1* had the highest frequency among the major *Vrn* genes in the

globally important CIMMYT wheat cultivars, with the semi-dwarf Mexican variety 'Sonora 64' as one of the varieties responsible for the wide distribution of *Vrn-D1* gene (van Beem et al., 2005).

Stelmakh (1998b) studied the F_2 generation of crosses among 647 randomly selected wheat cultivars and a set of near isogenic lines of *Vrn* genes in Triple Dirk background. He reported monogenic dominant *Vrn-A1* genotype in about 25% of global spring wheat cultivars. One half of the spring cultivars possessed both *Vrn-A1* and *Vrn-B1* in their genotype while only four lines had the three dominant genes *Vrn-A1*, *Vrn-B1* and *Vrn-D1* in combination. The *Vrn-D1* was observed less frequently in combination with *Vrn-A1* and/or *Vrn-B1*. Of the 45 spring cultivars from USA and Canada, 91% had *Vrn-A1*, 60% *Vrn-B1* and about 7% had *Vrn-D1*. Stelmakh (1993, 1998b) reported that the three major *Vrn* genes have differential effect on heading time, plant height and yield components. Genotypes having two dominant alleles in combination at two vernalization loci tended to mature early with high yield. The triple dominant genotypes were found to be early but low yielding. This suggests the possibility of combining specific dominant genes in spring wheats to improve grain yield potential while maintaining their earliness. Incorporation of *Vrn-D1* has been recommended in many spring wheat breeding programs.

Besides days to heading, other agronomic traits of importance are also affected by homoeologous group 5 chromosomes (Snape et al., 1985; Miura et al., 1992). Kato et al. (2001) reported that the presence of *Vrn-D1* gene in spring wheat lines reduced the number of spikelets spike^{-1} compared to those without this gene. Gonzalez et al. (2002) reported an increase in spikelet initiation rate in vernalization sensitive cultivars at vernalization saturation, while Rahman (1980) observed a decrease in spikelet initiation rate with vernalization. Duration of the stem elongation phase is also affected by vernalization under less inductive photoperiods (Gonzalez et al., 2002; 2003). It has also been observed that spikelet fertility at anthesis considerably increases with an extended period of stem elongation under field (Gonzalez et al., 2003) and controlled growth conditions (Miralles et al., 2000).

1.5.2. Photoperiod response

Photoperiod response is the second important genetic system determining flowering time, and hence adaptation of wheat to different agro-climatic conditions. As mentioned previously, vernalization is the primary factor determining winter and spring growth habit. However, flowering time of autumn sown spring or winter wheat varieties is not greatly affected by the presence of different *Vrn* genes, as their vernalization requirement is generally fulfilled (Worland & Snape, 2001). Under such conditions, flowering time is determined mainly by sensitivity/insensitivity to photoperiod (day length). A photoperiod insensitive variety immediately switches to reproductive growth with a rise in temperature in the spring, whereas a photoperiod sensitive variety continues its vegetative phase until the day length sufficiently increases to satisfy its photoperiod requirement (Worland & Snape, 2001). A lack of fulfillment of photoperiod requirement in sensitive varieties results in delayed flowering, the magnitude being determined by the presence of specific photoperiod response genes and the latitude of the growing region.

The response of different genotypes to photoperiod is genetically controlled by a series of photoperiod (*Ppd*) genes located on homoeologous group 2 chromosomes of wheat (Worland & Snape, 2001). To date, three such genes have been identified, including *Ppd-A1*, *Ppd-B1* and *Ppd-D1* (formerly *Ppd3*, *Ppd2* and *Ppd1*, respectively), located on chromosomes 2A, 2B and 2D, respectively (Law et al., 1978; Scarth & Law, 1983). Similar to *Vrn* gene system, dominant alleles of *Ppd* genes confer day length insensitivity, whereas the presence of recessive alleles results in day length sensitivity (Pugsley, 1966). Besides the major *Ppd* genes located on group 2 chromosomes, other chromosomes have been reported to affect heading time of wheat. Among these are the homoeologous group 1 chromosomes which delay heading in response to both vernalization and photoperiod (Law et al., 1998), and a photoperiod insensitive gene located on chromosome 3D (Miura & Worland, 1994). *Ppd-D1* is the most photoperiod insensitive locus followed by *Ppd-B1* and *Ppd-A1* (Worland, 1996).

Photoperiod response genes play a key role in accelerating or delaying heading time under field conditions in spring-sown cultivars that have been vernalized (Snape et

al., 2001). Photoperiod plays a more important role in flowering of vernalization insensitive spring wheats than that of winter wheats which respond to photoperiods only after their vernalization requirement is satisfied (Levy & Peterson, 1972; Davidson et al., 1985). However, photoperiod does not greatly affect the flowering time of spring wheat grown in higher northern latitudes, as the day length is long enough (>14 hours) to satisfy photoperiod requirements (Marshall et al., 1989; Worland et al., 1998; Kosner & Pankova, 1998). Mexican wheats are known for their wide adaptation to diverse climatic conditions partly due to their low sensitivity to photoperiod (Syme, 1968).

Law et al. (1978) reported a significant effect of homoeologous group 2 chromosomes on days to heading when they grew alien chromosome substitution lines of 'Chinese Spring' under different day lengths. Scarth and Law (1983) identified the photoperiod gene *Ppd-B1* on chromosome 2B of wheat by genetically analyzing recombinant inbred lines obtained by crossing 'Chinese Spring' with one of its substitution line for days to heading. Earlier, Keim et al. (1973) studied the inheritance of photoperiod response in crosses derived from photoperiod-sensitive and insensitive wheats, and suggested the presence of a 'two gene system' controlling heading time in response to different day lengths.

Studies examining the geographical distribution of *Ppd* genes indicated that winter wheat varieties grown in more northern latitudes (Canada, France, UK) were highly sensitive, whereas those grown in more southern latitudes (Italy, Yugoslavia) were highly insensitive to photoperiod (Martinic, 1975; Hunt, 1979). Within Europe, winter wheat varieties bred in southern regions were highly photoperiod insensitive, whereas those bred in northern regions were highly sensitive (Worland et al., 1994). Among spring wheats, highly sensitive genotypes have been found in a range of northern and southern latitudes (Martinic, 1975). However, most genotypes from southern latitudes were photoperiod insensitive. Kosner & Zurkova (1996) observed that wheat genotypes from lower geographical latitudes were less sensitive, whereas those from higher latitudes were more sensitive to photoperiod. They pointed out that wheat breeders have probably

indirectly chosen the most suitable *Ppd* genotypes for their growing regions simply by selecting for agronomically important traits.

In North America, the increased use of winter nurseries in southern latitudes as a means to accelerate breeding programs has lead to the development of more photoperiod insensitive cultivars (Dyck et al., 2004). However, photoperiod sensitivity has been found to be advantageous in the higher latitudes of North America in terms of yield stability, local adaptation and high productivity (Busch et al., 1984; Knott, 1986; Dyck et al., 2004). This area needs further investigation before making general recommendations regarding the utilization of particular photoperiod response mechanisms in wheat breeding programs in these regions.

In Europe, most day length insensitive wheat varieties carry a *Ppd-D1* allele that seems to be derived from the Japanese variety 'Akakomugi' or the related 'Saitama 27' (Worland, 1996). In a series of experiments conducted over a number of years in England, Germany and Yugoslavia, Worland (1996) reported that *Ppd-D1* affected ear emergence time, and exhibited pleiotropic effects on a number of agronomic traits. Results of these experiments indicated that *Ppd-D1* accelerated heading by 4 to 8 days. This shortened life cycle due to *Ppd-D1*, in turn reduced number of tillers, plant height and number of spikelets per ear. A positive effect of this gene was an increase in fertile spikelets, leading to high grain set per ear. Compared to photoperiod sensitive varieties, the photoperiod insensitive ones had 30% higher grain yield in southern Europe, 15% in mainland regions but no yield advantage in the UK. Pleiotropic effects of *Ppd-D1* on height reduction were greater than the effect of the dwarfing gene *Rht8* (Worland, 1996).

Pleiotropic effects of *Ppd-B1* on various agronomic traits have also been studied using chromosome 2B recombinant substitution lines (Worland et al., 1998). This gene also reduces heading time, tiller number, plant height and spikelet number, but the effects seem to be less pronounced than those of *Ppd-D1*. Lines with *Ppd-B1* yielded 7% greater than those having *Ppd-D1*, over a period of three years (Worland et al., 1998). Worland

and Snape (2001) suggested that photoperiod insensitivity is necessary for wheat cultivated under short day lengths.

Stelmakh (1998) studied the response of isogenic lines of the highly photosensitive winter wheat cultivar Mironovskaya 808 under different day lengths. Genotypes with dominant photoperiod insensitive gene(s) flowered early under long days compared to those having fully recessive sensitive gene(s). *Ppd-A1* resulted in maximum acceleration and minimum delay in heading time under short day lengths. Carriers of this gene had a greater number of spikelets and grain weight. In winter wheats, dominant photoperiod insensitive alleles decreased vernalization requirements. Days to heading decreased in spring cultivars grown under long days but increased under short days (Kosner & Zurkova, 1996).

Photoperiod affects both vegetative and reproductive phases of wheat, including the period from terminal spikelet initiation to heading (stem elongation) (Miralles & Richards, 2000). Slafer et al. (1996) suggested that extending the stem elongation phase in the life cycle of wheat could increase spike growth, leading to higher grain yield. Both fertile spikelets and spike dry weight at anthesis increased with lengthening duration of late reproductive phase under field conditions, possibly due to the partitioning of increased assimilates to the spike compared to the stem (Gonzalez et al., 2003a). Miralles et al. (2000) reported that increased spikelet fertility was associated with longer duration of the late reproductive phase under controlled growth conditions. Close association of spikelet fertility with stem elongation has been suggested to be a means of improving the yield potential of wheat, as grain number per spike is usually determined by spikelet fertility (Slafer et al., 1996).

Gonzalez et al., (2003b) studied the mechanisms responsible for greater partitioning of assimilates to the spike at anthesis as a result of lengthened growth period of the spike. They reported that an extended stem elongation period in response to short day lengths actually increased duration of spike growth but did not affect spike growth rate. They suggested that with an increased stem elongation period, competition between

stem and spike for assimilates is reduced, resulting in greater partitioning of assimilates to the spike (Gonzalez et al., 2003a). Generalizing this phenomenon to day length sensitive wheat cultivars could suggest that an early shift to reproductive phase would favor dry matter partitioning to spike under short days.

1.5.3. Earliness *per se*

Varietal differences in flowering time other than those due to photoperiod and vernalization response have been observed in wheat (Halloran, 1975; Ford et al., 1981; Scarth & Law, 1983). Such genetic differences are termed "earliness *per se*" (Hoogendoorn, 1985), 'narrow-sense earliness' (Kato et al., 1998) or 'intrinsic earliness' (Slafer, 1996). Earliness *per se* is the difference in flowering times of plants whose vernalization and photoperiod requirements have been fulfilled (Kato et al., 2001). Photoperiod and vernalization response genes determine flowering time of wheat in response to specific day length and temperature, whereas earliness *per se* genes control flowering time independent of environmental stimuli (Worland, 1996). Major vernalization and photoperiod genes may be regarded as 'modifiers of earliness' because they influence flowering only in response to certain environmental conditions, but earliness is determined by a minimum vegetative growth that can initiate floral primordia independent of external stimuli (Kato & Wada, 1999).

Because of their major effects on flowering time, vernalization and photoperiod response genes have been studied in detail, while earliness *per se* genes have not been fully investigated (Kato & Wada, 1999). Earliness *per se* is a quantitatively inherited trait controlled by a number of minor genes whose effects can be determined only in the absence of the confounding effects of vernalization and photoperiod response genes (Kato & Wada, 1999). Earliness *per se* is highly heritable and can, therefore, be effectively utilized in breeding programs to shorten wheat's life cycle independent of other environmental factors known to modify flowering time (Kato & Wada, 1999).

The first earliness *per se* (*eps*) gene was reported by Scarth and Law (1983) on the long arm of chromosome 2B affecting ear emergence time in recombinant inbred

lines of 'Chinese Spring', and one of its chromosome substitution line. Miura and Worland (1994) grew vernalized plants of group 3 chromosome substitution lines in 'Chinese Spring' background under long days, and observed that most of the substitution lines flowered earlier than the parent 'Chinese Spring' cultivar. This was accompanied by reduced leaf and spikelet number. It was concluded that chromosome 3A carried an earliness *per se* allele. Kato et al. (1999) identified a quantitative trait locus (QTL) for earliness *per se* on the proximal end of chromosome 5AL of single chromosome substitution lines. Miura et al. (1999) studied flowering times of ditelosomic and recombinant substitution lines of 'Chinese Spring' with chromosome 3A from 'Timstein'. Based on the differences in flowering times they proposed that both the short and long arms of chromosome 3A carry "earliness *per se*" genes. Working with deletion lines of group 5 chromosomes of wheat, Sarma et al. (2000) mapped an "earliness *per se*" gene together with *Vrn-A1* and grain hardness (Ha) gene on chromosome 5A. Shindo et al. (2003) detected an earliness *per se* QTL close to *Ppd-B1* on chromosome 2B. Toth et al. (2003), using single chromosome recombinant inbred line population, identified a genomic region on the long arm of chromosome 5B affecting earliness *per se*. More recently, Hanocq et al. (2004) detected four earliness *per se* QTL, one each on chromosomes 2B, 2D, 5B and 7A, which together accounted for about 27-29% of the variation in the heading time of a recombinant inbred population.

In the genetic analyses of flowering time, earliness *per se* genes tend to have relatively smaller effects, and are generally mapped as QTL rather than as major genes (Kato et al., 1999). This is partly because most of the earliness *per se* QTL have been identified in mapping populations developed for locating major vernalization or photoperiod response genes (Kato et al., 1999). Detailed study of earliness *per se* genes require precise genetic stocks and environmental conditions, where the presence of such genes is not obscured by the effects of *Vrn* and *Ppd* genes. Earliness *per se* genes have been found to influence flowering time of wheat in many studies but none of these reported that *eps* genes have pleiotropic effects on any other traits of agronomic importance (Worland & Snape, 2001). However, due to their independent nature, such

genes could be of value to wheat breeders in modifying flowering time, further adding to the wide adaptability of wheat.

1.6. Molecular approaches to study the genetics of bread wheat

Recent progress in the area of plant molecular biology and genomics has greatly improved our understanding of the genetic makeup of living organisms. The capacity of the hexaploid wheat genome to tolerate the addition or loss of whole or parts of chromosome(s) facilitated the development of special genetic stocks which resulted in rapid progress in early wheat genetics using cytogenetic techniques (Lagudah et al., 2001). However, compared to maize, rice or tomato, developments in wheat genomics have been slow due to its higher ploidy level, the size and complexity of the genome, the presence of highly repetitive DNA sequences and low levels of polymorphism (Hoisington et al., 2002). Nevertheless, the development of molecular techniques for genetic analysis, especially the use of molecular markers for detecting/monitoring differences in DNA sequence between varieties, landraces and wild relatives of wheat, and related grass species has dramatically expanded our understanding of wheat genetics (Langridge et al., 2001). Moreover, the existence of genetic similarities among eukaryotic genomes provides the basis for using model plant systems like *Arabidopsis* and rice to study the structure and organization of larger genomes such as wheat, in an approach known as comparative genomics (Lagudah et al. 2001).

1.6.1. Molecular (DNA) markers

A DNA marker is a small region of DNA exhibiting sequence polymorphism in different individuals within a species or group of individuals. Molecular markers can be used for the development of detailed genetic and physical chromosome maps, as an indirect selection tool for both simple and quantitative traits to improve selection efficiency, for germplasm characterization, for studying the structure and function of genomes, and in phylogenetic analysis etc. (Gupta et al., 1999). The use of molecular markers as an indirect selection tool in breeding programs, known as marker assisted selection (MAS), offers a great advantage over conventional methods because these markers are not influenced by environment and can be scored at all stages of plant growth

(Gupta et al., 1999). In order to be of practical value in breeding programs, a marker must be closely linked to the gene controlling the trait of interest, must show polymorphism amongst the parents so that the desirable allele can be distinguished from the undesirable one, and must be cost-effective (Eagles et al., 2001).

1.6.2 Use of DNA markers to study flowering time in wheat

Several types of DNA markers are available, each having some advantages and disadvantages. For a complete description of all marker types the reader is referred to Gupta et al. (1999) and Collard et al. (2005).

Galiba et al. (1995) and Kato et al. (1998) mapped the *Vrn-A1* gene on the long arm of chromosome 5A of wheat using restriction fragment length polymorphism (RFLP) markers. The vernalization gene *Vrn-B1* has been mapped on the long arm of chromosome 5B using microsatellite markers (Leonova et al., 2003; Toth et al., 2003). Molecular markers linked to *Vrn-B1* have been identified by Salina et al. (2003) using microsatellites and by Barrett et al. (2002) using amplified fragment length polymorphisms (AFLPs) and microsatellite markers. The vernalization gene *Vrn-D1* has been mapped to chromosome 5DL using microsatellites (Snape et al., 2001b). Hanocq et al. (2004) detected a major QTL for vernalization response on chromosome 5A using microsatellites.

Worland (1996) and Worland et al. (1998) mapped the major photoperiod genes, *Ppd-B1* and *Ppd-D1* on chromosomes 2B and 2D, respectively using RFLP markers. *Ppd-B1* has also been recently mapped using AFLPs and microsatellites (Mohler et al., 2004). Hanocq et al. (2004) detected two major photoperiod sensitive QTL on chromosomes 2B and 2D, close to the two major photoperiod response genes *Ppd-B1* and *Ppd-D1*. They also reported two other QTL affecting photoperiod sensitivity on chromosomes 5A and 7D.

Earliness *per se* quantitative trait loci (QTL) have been detected by Kato et al. (1999) and Kato et al. (2002) on chromosome 5AL of wheat using RFLP markers. Toth

et al. (2003) also identified an earliness *per se* QTL on chromosome 5BL using microsatellites. Shindo et al. (2003) detected two earliness *per se* QTL on chromosome 2B using RFLPs. Hanocq et al. (2004) also reported the presence of earliness *per se* QTL on chromosomes 2B and 2D using microsatellites.

1.6.3. Bulked segregant analysis and selective genotyping

Mapping of QTL relies on the construction of complete linkage maps of populations segregating for the trait of interest and QTL analysis, both taking considerable amount of time, effort and money. Two shortcut methods (bulked segregant analysis and selective genotyping) are available that can provide rapid and cost-effective approaches for the identification of markers linked to QTL (Collard et al., 2005).

Bulked segregant analysis (BSA) was developed by Michelmore et al. (1991), and involves pooling individuals from the two phenotypic extremes of the target trait distribution in a population derived from parents differing in the trait of interest. DNA isolated from an equal number of individuals within each extreme is then bulked and subsequently screened with DNA markers. Polymorphic markers are derived from genomic regions that are different between the two pools with the remainder of the genome randomly contributed by the parents. The entire mapping population may then be genotyped with the polymorphic markers to generate linkage maps. BSA is generally used to map genes controlling simply inherited traits, but is also useful for identifying markers linked to QTL (Langridge et al., 2001).

Selective genotyping (also known as 'trait-based marker analysis') involves genotyping of individuals from the phenotypic extremes of a mapping population (Lander & Botstein, 1989). In this approach, linkage map construction and QTL analysis is performed using only a number of individuals from the phenotypic extremes. Significant difference in the allelic frequency at any marker locus between the two extremes indicates the presence of a QTL near the particular marker. Selective genotyping greatly reduces the costs of genotyping in mapping studies. However, it is not efficient in

determining the effect of individual QTLs, and can not be used for more than one trait at a time (Tanksley, 1993).

1.7. Conclusions

Bread wheat is the most widely grown crop in the world due to its ability to survive under different eco-geographical conditions. The wide adaptability of wheat to diverse environments is mainly due to the exploitation of genes controlling time of flowering and maturity. Genetic control of flowering time in wheat is complex, being influenced by three major groups of genes which include vernalization and photoperiod response, and earliness *per se* genes. Vernalization and photoperiod genes have major effects on flowering time and act in response to the environmental stimuli of cold temperatures and day length. Earliness *per se* genes modify flowering time independently of environmental stimuli and have minor effects. These three sets of genes act together to determine the appropriate flowering time, thereby the suitability of a given genotype to particular environmental conditions. Besides influencing flowering time, vernalization and photoperiod response genes have pleiotropic effects on other aspects of plant's growth and development with respect to grain yield potential. The geographical distribution of photoperiod and vernalization genes has demonstrated that these genes have different breeding values for a given set of environmental conditions. Progress in molecular genetics, especially the availability of highly polymorphic markers, has made it possible to monitor genomic regions affecting important economic traits in wheat. The characterization of individual genes and the identification of diagnostic markers for the key photoperiod, vernalization and earliness *per se* genes may make it possible to 'fine tune' flowering time of wheat, further adding to the wide adaptability of this crop.

1.8. Objectives

Early maturity is an important objective in spring wheat breeding programs in higher northern latitudes such as western Canada. Earliness is required due the short growing season (95-125 d) and potential for preharvest sprouting of physiologically mature grains. Presently, there is a limited knowledge of the specific genes controlling flowering/maturity times of spring wheats grown in western Canada. It is, therefore, very

important that wheat breeders in these regions know the genetic basis of flowering/maturity times before formulating strategies for the development of early maturing spring wheat cultivars. Such knowledge is likely to aid in breeding early maturing spring wheat cultivars with higher grain yield potential. Keeping these facts in mind, the present set of studies were conducted to:

1) Investigate the relative importance of the three genetic factors (vernalization, photoperiod and earliness *per se*) that determine flowering/maturity times in spring wheats grown in western Canada.
2) Study the inheritance of flowering/maturity times in western Canadian adapted spring wheats under controlled environmental and field conditions.
3) Identify different vernalization genes in selected Canadian spring wheat cultivars.
4) Study the effects of different vernalization gene(s) on flowering/maturity times, and other important agronomic traits under different planting time.
5) Investigate the association of time to maturity with grain yield and grain protein in a large population of early maturing spring wheat lines/cultivars.

The specific null hypotheses tested in the following chapters were:

1) Vernalization, photoperiod and earliness *per se* genes are equally important in controlling flowering/maturity time in western Canadian spring wheat.
2) Flowering/maturity time differences in western Canadian adapted spring wheats are not genetically controlled.
3) Canadian spring wheat cultivars do not carry different vernalization genes.
4) Vernalization genes do not affect flowering/maturity times and other important agronomic traits under different planting time.
5) There is no association of time to maturity with grain yield and grain protein content in spring wheat.

Results of these investigations should contribute to the better understanding of the genetic basis of flowering/maturity time in western Canadian spring wheats in particular, and to the association of maturity with grain yield and protein content in general.

1.9 References

Barrett, B., M. Bayram, and K. Kidwell. 2002. Identifying AFLP and microsatellite markers for vernalization response gene *Vrn-B1* in hexaploid wheat using reciprocal mapping populations. Plant Breed. 121: 400-406.

Berry, G.J., P.A. Salisbury, and G.M. Halloran. 1980. Expression of vernalization genes in near-isogenic wheat lines. Duration of vernalization period. Ann. Bot. 46: 235-241.

Bootsma, A., and R. DeJong. 1988. Estimates of seeding dates of spring wheat on the Canadian prairies from climate data. Can. J. Plant Sci. 68: 513-517.

Briggle, L.W., and B.C. Curtis. 1987. Wheat worldwide. In: E.G. Heyne (ed.) *Wheat and wheat improvement*. Am. Soc. Agron., Madison, W.I., pp.1-31.

Briggs, K.G., A.M. Johnson-Flangan, and A. Laroche. 1998. Using Cadet/Rescue 5A, 5B and 5D chromosome substitution series to verify, locate and tag genes for early heading and maturity, including vernalization genes. In: *Proceedings of the 9th Inter. Wheat Genet. Symp.*, University of Saskatchewan, SK, Vol. 2, pp.19-21.

Brooking, I.R. 1996. Temperature response of vernalization in wheat: A developmental analysis. Ann. Bot. 78:507-512.

Bruckner, P.L., and R.C. Frohberg. 1987. Rate and duration of grain fill in spring wheat. Crop Sci. 27: 451-455.

Chouard, P. 1960. Vernalization and its relation to dormancy. Ann. Rev. Plant Physio. 11: 191-237.

Collard, B.C.Y., M.Z.Z. Jahufer, J.B. Brouwer, and E.C.K. Pang. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica, 142: 169-196.

Cooper, J.P. 1956. Developmental analysis of populations in the cereals and herbage grasses. 1. Methods and techniques. J. Agric. Sci. 47: 262-279.

Canada Grains Council. 2001. Statistical handbook.

Candlish, B. 2002. Wheat other than Durum (Variety). [Online] Available: http://members.shaw.ca/bethcandlish/swht.htm [24 October 2003].

Davidson, J.L., K.R. Christian, D. B. Jones, and P.M. Bremner. 1985. Responses of wheat to vernalization and photoperiod. Aus. J. Agric. Res. 36: 347-359.

Darroch, B.A., and R.J. Baker. 1990. Grain filling in three spring wheat genotypes: Statistical analysis. Crop Sci. 30: 525-529.

Darroch, B.A., and R.J. Baker. 1995. Two measures of grain filling in spring wheat. Crop Sci. 35: 164-168.

DePauw, R.M., G.R. Boughton, and D.R. Knott. 1995. Hard spring wheat. In: Harvest of Gold-The history of field crop breeding in Canada; A.E. Slinkard and D.R. Knott (eds.), University of Saskatchewan Press, Saskatoon, Saskatchewan, pp. 5-35.

Dubcovsky, J., D. Lijavetzky, L. Appendino, D. Tranquilli, and J.D. Dvorak. 1998. Comparative RFLP mapping of *Triticum monococcum* genes controlling vernalisation requirement. Theor. Appl. Genet. 97: 968-975.

Duguid, S.D., and A.L. Brule-Babel. 1994. Rate and duration of grain filling in five spring wheat (Triticum aestivum L.) genotypes. Can. J. Plant Sci. 74: 681-686.

Dyck, J.A., M.A. Matus-Cadiz, P. Hucl, L. Talbert, T. Hunt, J.P. Dubuc, H. Nass, G. Clayton, J. Dobb, and J. Quick, 2004. Agronomic performance of hard red spring wheat isolines sensitive and insensitive to photoperiod. Crop Sci. 44: 1976-1981.

Eagles, H.A., H.S. Bariana, F.C. Ogbonnaya, G.J. Rebetzke, G.J. Hollamby, R.J. Henry, P.H. Henschke, and M. Carter. 2001. Implementation of markers in Australian wheat breeding. Aust. J. Agric. Res. 52: 1349-1356.

Flood, R.G., and G.M. Halloran. 1984. The nature and duration of gene action for vernalization response in wheat. Ann. Bot. 53: 363-368.

Flood, R.G., and G.M. Halloran. 1986. The influence of genes for vernalization response on development and growth in wheat. Ann. Bot. 58: 505-513.

Ford, M.A., R.B. Austin, W.J. Angus, and G.C.M. Sage. 1981. Relationship between the responses of spring wheat genotypes to temperature and photoperiodic treatments and their performance in the field. J. Agric. Sci. 96: 623-634.

Gebeyehou, G., D.R. Knott, and R.J. Baker. 1982. Relationships among durations of vegetative and grain filling phases, yield components, and grain yield in durum wheat cultivars. Crop Sci. 22: 287-290.

Goncharov, N.P. 1998. Genetic resources of wheat related species: The *Vrn* genes controlling growth habit (spring vs winter). Euphytica, 100: 371-376.

Goncharov, N.P. 2003. Genetics of growth habit (spring vs winter) in common wheat: confirmation of the existence of dominant gene *Vrn4*. Theor. Appl. Genet. 107: 768-772.

Goncharov, N.P. 2004. Response to vernalization in wheat: its quantitative or qualitative nature. Cereal Res. Commun. 32: 323-330.

Gonzalez, F.G., G.A. Slafer, and D.J. Miralles. 2002. Vernalization and photoperiod responses during wheat pre-flowering reproductive phases. Field Crops Res. 74: 183-195.

Gonzalez, F.G., G.A. Slafer, and D.J. Miralles. 2003a. Grain and floret numbering response to photoperiod during stem elongation in fully and slightly vernalized wheats. Field Crops Res. 81: 17-27.

Gonzalez, F.G., G.A. Slafer, and D.J. Miralles. 2003b. Floret development and spike growth as affected by photoperiod during stem elongation in wheat. Field Crops Res. 81: 29-38.

Gororo, N.N., R. G. Flood, R.F. Eastwood, and H.A. Eagles. 2001. Photoperiod and vernalization responses in *Triticum turgidum* x *T. tauschii* synthetic hexaploid wheats. Ann. Bot. 88:947-952.

Gotoh, T. 1980. Gene analysis of the degree of vernalization requirement in winter wheat. Japan J. Breed. 30: 1-10.

Gupta, P.K., P.L. Kulwal, and S. Rustgi. 2005. Wheat cytogenetics in the genomics era and its relevance to plant breeding. Cytogenet. Genome Res. 109: 315-327.

Gupta, P.K., R.K. Varshney, P.C. Sharma, and B. Ramesh. 1999. Molecular markers and their applications in wheat breeding. Plant Breed. 118: 369-390.

Halloran, G.M. 1975. Genotype differences in photoperiodic sensitivity and vernalization response in wheat. Ann. Bot. 39: 845-851.

Hanocq, E., M. Niarquin, E. Heumez, M. Rousset, and J. Le Gouis. 2004. Detection and mapping of QTL for earliness components in a bread wheat recombinant inbred lines population. Theor. Appl. Genet. 110: 106-115.

Hoisington, D., N. Bohorova, S. Fennel, M. Khairallah, A. Pellegrineschi, and J.M. Ribaut. 2002. The application of biotechnology to wheat improvement. In: B.C.

Curtis, S. Rajaram & H.G. Macpherson (eds.) *Bread wheat: Improvement and production*. FAO, Italy. Pp. 175-198.

Hoogendoorn, J. 1985. The basis of variation in date of ear emergence under field conditions among the progeny of a cross between two winter wheat varieties. J. Agric. Sci. 104: 493-500.

Hucl, P., and Matus-Cadiz, M. 2002. CDC EMDR-4, CDC EMDR-9, and CDC EMDR-14 spring wheats. Can. J. Plant Sci. 82: 411-413.

Hunt, L.A. 1979. Photoperiodic responses of winter wheats from different climatic regions. J. Plant Breed. 82: 70-80.

Islam-Faridi, M.N., A.J. Worland, and N.C. Law. 1996. Inhibition of ear-emergence time and sensitivity to day length determined by the group 6 chromosomes of wheat. Heredity, 77:572-580.

Iwaki, K., and K. Kato. 1998. Geographical variation of *Vrn* genotype in wheat caused by the selection for adaptability to winter coldness. In: *Proceedings of the 9th Inter. Wheat Genetics Symp*. University of Saskatchewan, 4: pp-39-41.

Jedel, P.E., L.E. Evans, and R. Scarth. 1986. Vernalization responses of a selected group of spring wheats (*Triticum aestivum* L.) cultivars. Can. J. Plant Sci. 66: 1-9.

Kato, K., and T. Wada. 1999. Genetic analysis and selection experiment for narrow-sense earliness in wheat by using segregating hybrid progenies. Breed. Sci., 49: 233-238.

Kato, K., H. Miura, and S. Sawada. 1999. Detection of an earliness per se quantitative trait locus in the proximal region of wheat chromosome 5AL. Plant Breed. 118: 391-394.

Kato, K., H. Miura, M. Akiyama, M. Kuroshima, and S. Sawada. 1998. RFLP mapping of the three major genes, *Vrn-A1*, Q and B1, on the long arm of chromosome 5A of wheat. Euphytica, 101: 91-95.

Kato, K., S. Taketa, T. Ban, N. Iriki, and K. Miura. 2001. The influence of a spring habit gene, *Vrn-D1*, on heading time in wheat. Plant Breed. 120: 115-120.

Kato, K., M. Yamashita, K. Ishimoto, H. Yoshino, and M. Fujita. 2003. Genetic analysis of two genes for vernalization response, the former *Vrn2* and *Vrn4*, by using PCR based molecular markers. In: Pogna, N.E., M. Romano, E.A. Pogna, and G.

Galterio (eds) Proceedings of the 10th International wheat genetics symposium , Instituto Sperimentale per la Cerealicoltura, Paestum, 3: 971-973.

Keim, D. L., J.R. Welsh, and R.L. McConnell. 1973. Inheritance of photoperiodic heading response in winter and spring cultivars of bread wheat. Can. J. Plant Sci. 53: 247-250.

Klaimi, Y.Y., and C.O. Qualset. 1974. Genetics of time of heading in wheat (*Triticum aestivum* L.) II. The inheritance of vernalization response. Genetics, 76: 119-133.

Kosner, J., and K. Pankova. 1998. The detection of allelic variants at the recessive vrn loci of winter wheat. Euphytica, 101:9-16.

Kosner, J., and D. Zurkova. 1996. Photoperiodic response and its relation to earliness in wheat. Euphytica, 89: 59-64.

Kuspira, J., J. Maclagan, K. Kirby, and R.N. Bhambhani. 1986. Genetic and cytogenetic analyses of the A genome of *Triticum monococcum* L. II. The mode of inheritance of spring versus winter growth habit. Can. J. Genet. Cytol. 28: 88-95.

Lagudah, E.S., J. Dubcovsky, and W. Powell. 2001. Wheat genomics. Plant Physiol. Biochem. 39: 335-344.

Lander, E. S., and D. Botstein. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics, 121: 185-199

Langridge, P., E.S. Lagudah, T.A. Holton, R. Appels, P.J. Sharp, and K.J. Chalmers. 2001. Trends in genetic and genome analysis in wheat: a review. Aust. J. Agric. Res. 52: 1043-1077.

Law, C.N., and R. Scarth. 1984. Genetics and its potential for understanding the action of light in flowering. In: Vince-Prue, D., B. Thomas, K.E. Cockshull (eds.) *Light and the flowering process*. Academic press, New York, 193-209.

Law, C.N., and M.S. Wolfe. 1966. Location of genetic factors for powdery mildew resistance and ear emergence time on chromosome 7B of wheat. Can. J. Genet. Cytol. 8: 462-470.

Law, C.N., and A.J. Worland. 1997. Genetic analysis of some flowering time and adaptive traits in wheat. New Phytol. 137: 19-28.

Law, C.N., J. Sutka, and A.J. Worland. 1978. A genetic study of day length response in wheat. Heredity, 41: 575-585.

Law, C.N., A.J. Worland, and B. Giorgi. 1976. The genetic control of ear emergence time by chromosomes 5A and 5D of wheat. Heredity, 36: 49-58.

Law, C.N., E. Suarez, T.E. Miller, and A.J. Worland. 1998. The influence of the group 1 chromosomes of wheat on ear-emergence times and their involvement with vernalization and day length. Heredity, 41: 185-191.

Levy, J., and M.L. Peterson. 1972. Responses of spring wheats to vernalization and photoperiod. Crop Sci. 12:487-490.

Major, D.J., and E.D.P. Whelan. 1985. Vernalization and photoperiod response characteristics of a reciprocal substitution series of rescue and Cadet hard red spring wheat. Can. J. Plant Sci. 65: 33-39.

Marshall, L., R. Busch, F. Cholick, I. Edwards, and R. Frohberg. 1989. Agronomic performance of spring wheat isolines differing for day length response. Crop Sci. 29: 752-757.

Martinic, Z.F. 1975. Life cycle of common wheat varieties in natural environments as related to their response to shortened photoperiod. [illegible]nt Breed. 75: 237-251.

McCaig, T.N., and DePauw, R.M. 1992. Breeding for pre-harvest sprouting tolerance in white seed coat spring wheat. Crop Sci. 32:19-25.

McIntosh, R.A., K.M. Devos, J. Dubcovsky, C.F. Morris, and W.J. Rogers. 2003. Catalogue of gene symbols for wheat. [Online] http://wheat.pw.usda.gov/ggpages/wgc/2003/GeneSymbol.html. [21 Apr 2006].

Miralles, D.J., and R.A. Richards. 2000. Responses of leaf and tiller emergence and primordium initiation in wheat and barley to interchanged photoperiod. Ann. Bot. 85: 655-663.

Miralles, D.J., R.A. Richards, and G.A. Slafer. 2000. Duration of stem elongation period influences the number of fertile florets in wheat and barley. Aus. J. Plant Physiol. 27:931-941.

Miura, H., and A.J. Worland. 1994. Genetic control of vernalization, day length response and earliness per se by homoeologous group 3 chromosomes in wheat. Plant Breed. 113: 160-169.

Miura, H., M. Nakagawa, and A.J. Worland. 1999. Control of ear emergence time by chromosome 3A of wheat. Plant breeding, 118: 85-87.

Miura, H., B.B. Parker, and J.W. Snape. 1992. The location of major genes and associated quantitative trait loci on chromosome arm 5BL of wheat. Theor. Appl. Genet. 85: 197-204.

Mohler, V., R. Lukman, S. Ortiz-Islas, M. William, A.J. Worland, J. van Beem, and G. Wenzel. 2004. Genetic and physical mapping of photoperiod insensitive gene *Ppd-B1* in common wheat. Euphytica, 138: 33-40.

Naranjo, T., and E. Corredor. 2004. Clustering of centromeres precedes bivalent chromosome pairing of polyploidy wheats. Trends Plant Sci. 9: 214-217.

Nass, H.G., and B. Reiser. 1975. Grain filling period and grain yield relationships in spring wheat. Can. J. Plant Sci. 55: 673-678.

Noll, J.S., P.L. Dyck, and E. Czarnecki. 1982. Expression of RL4137 type of dormancy in F_1 seeds of reciprocal crosses in common wheat. Can. J. Plant Sci. 62:345-349.

Ortiz Ferrara, G., M.G. Mossad, V. Mahalakshmi, and S. Rajaram. 1998. Photoperiod and vernalisation response of Mediterranean wheats, and implications for adaptation. Euphytica, 100: 377-384.

Poehlman, J.M., and D.A. Sleper. 1995. Breeding Field Crops. Iowa State University press, Ames, Iowa, p-151.

Pugsely, A.T. 1966. The photoperiodic sensitivity of some spring wheats with special reference to the variety Thatcher. Aust. J. Agric. Res. 17: 591-599.

Pugsely, A.T. 1971. A genetic analysis of the spring wheat habit of growth in wheat. Aust. J. Agric. Res. 22:23-31.

Pugsely, A.T. 1972. Additional genes inhibiting winter habit in wheat. Euphytica, 21: 547-552.

Rahman, M.S. 1980. Effect of photoperiod and vernalization on the rate of development and spikelet number per ear in 30 varieties of wheat. J. Aus. Inst. Agric. Sci. 46:68-70.

Riley, R., and V. Chapman. 1958. Genetic control of the cytologically diploid behavior of hexaploid wheat. Nature, 182: 713-715.

Roberts, D.W.A., and R.I. Larson. 1985. Vernalization and photoperiod responses of selected chromosome substitution lines derived from 'Rescue', 'Cadet' and 'Cypress' wheats. Can. J. Genet. Cytol. 27: 586-591.

Salina, E., O. Dobrovolskaya, T. Efremova, I. Leonova, and M.S. Roder. 2003. Microsatellite monitoring of recombination around the Vrn-B1 locus of wheat during early backcross breeding. Plant Breed. 122:116-119.

Sarma, R.N., L. Fish, B.S. Gill, and J.W. Snape. 2000. Physical characterization of the homoeologous group 5 chromosomes of wheat in terms of rice linkage blocks, and physical mapping of some important genes. Genome, 43: 191-198.

Scarth, R., and C.N. Law. 1983. The location of the photoperiod gene *Ppd-B1* and an additional genetic factor for ear emergence time on chromosome 2B of wheat. Heredity, 51: 607-619.

Scarth, R., and C.N. Law. 1984. The control of day length response in wheat by the group 2 chromosomes. J. Plant Breed. 92: 140-150.

Sharma, R.C. 1994. Early generation selection for grain-filling period in wheat. Crop Sci. 34: 945-948.

Shindo, C., H. Tsujimoto, and T. Sasakuma. 2003. Segregation analysis of heading traits in hexaploid wheat utilizing recombinant inbred lines. Heredity, 90: 56-63.

Slafer, G.A. 1996. Differences in phasic development rate amongst wheat cultivars independent of responses to photoperiod and vernalization. A view point of the intrinsic earliness hypothesis. J. Agric. Sci. 126: 403-419.

Slafer, G.A., and H.M. Rawson. 1994. Sensitivity of wheat phasic development to major environmental factors: a re-examination of some assumptions made by physiologists and modelers. Aus. J. Plant Physiol. 21: 393-426.

Slafer, G.A., D.F. Calderini, and D.J. Miralles. 1996. Yield components and compensation in wheat: opportunities for further increasing yield potential. In: Reynolds, M.P., S. Rajaram, and A. McNab (eds.), Increasing yield potential in wheat: breaking the barriers, CIMMYT, pp. 101-133.

Snape, J.W., C.N. Law, B.B. Parker, and A.J. Worland. 1985. Genetical analysis of chromosome 5 A of wheat and its influence on important agronomic characters. Theor. Appl. Genet. 71: 518-526.

Snape, J.W., K. Butterworth, E.M. Whitechurch, and A.J. Worland. 2001a. Waiting for fine times: genetics of flowering time in wheat. Euphytica, 119: 185-190.

Snape, J.W., R. Sarma, S.A. Quarrie, L. Fish, G. Galiba, and J. Sutka. 2001b. Mapping genes for flowering time and frost tolerance in cereals using precise genetic stocks. Euphytica, 120: 309-315.

Sofield, I., L.T. Evans, M.G. Cook, and I.F. Wardlaw. 1977. Factors influencing the rate and duration of grain filling in wheat. Aust. J. Plant Physiol. 4: 785-797.

Statistics Canada. 2001. The Daily: Production of principal field crops. http://www.statcan.ca.

Stelmakh, A.F. 1993. Genetic effect of Vrn genes on heading date and agronomic traits in bread wheat. Euphytica, 65:53-60.

Stelmakh, A.F. 1998a. Genetic regulation of ontogenetic rate as the valid base of yield stabilization. In: R.K. Behl, D.P. Singh & G.P. Lodhi (eds.) *Crop improvement for stress tolerance*. CCSHAU, Hisar & MMB, New Delhi. Pp. 102-117.

Stelmakh, A.F. 1998b. Genetic systems regulating flowering response in wheat. Euphytica, 100: 359-369.

Sutka, J., G. Galiba, A. Vagujfalvi, B.S. Gill, and J.W. Snape. 1999. Physical mapping of the *Vrn-A1* and *Fr1* Genes on chromosome 5A of wheat using deletion lines. Theor. Appl. Genet. 99: 199-202.

Syme, J.R. 1968. Ear emergence of Australian, Mexican and European wheats in relation to time of sowing and their response to vernalization and day length. Aus. J. Exp. Agric. Anim. Husb. 8: 578-581.

Talbert, L. E., S. P. Lanning, R. L. Murphy, and J. M. Martin. 2001. Grain Fill Duration in Twelve Hard Red Spring Wheat Crosses: Genetic Variation and Association with Other Agronomic Traits. Crop Sci. 41: 1390-1395.

Tames, S. 2005. Cultivar development. [Online] http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/crop12751 [20 Apr 2006].

Tanksley, S.D. 1993. Mapping polygenes. Annu. Rev. Genet. 27: 205-233.

Toth, B., G. Galiba, E. Feher, J. Sutka, and J.W. Snape. 2003. Mapping genes affecting flowering time and frost resistance on chromosome 5B of wheat. Theor. Appl. Genet. 107: 509-514.

Van Beem, J., V. Mohler, R. Lukman, M. van Ginkel, M. William, J. Crossa, and A.J. Worland, 2005. Analysis of genetic factors influencing the developmental rate of globally important CIMMYT wheat cultivars. Crop Sci. 45: 2113-2119.

Wang, H., T.N. McCaig, R.M. DePauw, F.R. Clarke, and J.M. Clarke. 2002. Physiological characteristics of recent CWRS wheat cultivars: Yield components and dry matter production. Can. J. Plant Sci. 82:299-306.

Wang, S.Y., R.W. Ward, J.T. Ritchie. R.A. Fisher, and U. Schulthess. 1995a. Vernalization in wheat I. A model based on the interchangeability of plant age and vernalization duration. Field Crops Res. 41: 91-100.

Wang, S.Y., R.W. Ward, J.T. Ritchie. R.A. Fisher, and U. Schulthess. 1995b. Vernalization in wheat II. Genetic variability for the interchangeability of plant age and vernalization duration. Field Crops Res. 44: 67-72.

Wong, L.S.L., and R.J. Baker. 1986a. Developmental patterns in five spring wheat genotypes varying in time to maturity. Crop Sci. 26: 1167-1170.

Wong, L.S.L., and R.J. Baker. 1986b. Selection for time to maturity in spring wheat. Crop Sci. 26:1171-1175.

Worland, A.J. 1996. The influence of flowering time genes on environmental adaptability in European wheats. Euphytica, 89: 49-57.

Worland, T., and J.W. Snape. 2001. Genetic basis of worldwide wheat varietal improvement. In: Bonjean, A.P. & W. J. Angus (eds.) *The World Wheat Book; A history of wheat breeding*. Intercept Ltd., UK, pp. 61-67.

Worland, A.J., M.L. Appendino, and E.J. Sayers. 1994. The distribution of genes influencing ecoclimatic adaptability in European winter wheat varieties. Euphytica, 80: 219-228.

Worland, A.J., A. Borner, V. Korzun, W.M. Li, S. Petrovic, and E.J. Sayers. 1998. The influence of photoperiod genes on the adaptability of European winter wheats. Euphytica, 100: 385-394.

Chapter 2

Genetic Control of Flowering and Maturity Time in Canadian Spring Wheat[2]

2.1 Introduction

Early maturity is an important objective of spring wheat breeding programs in northern growing regions. Short growing seasons, low temperatures early and late in the growing season, and long days (>14 hours) typify regions such as the Canadian prairies. Due to the short growing season (95-125 days) in western Canada, the development of early maturing cultivars is important to avoid frost damage which can adversely affect production and quality. Early maturing cultivars may also be less prone to pre-harvest sprouting (Hucl & Matus-Cadiz, 2002) that is common in years of cold and wet harvest conditions.

Growth and developmental phases (tillering, stem elongation, ear emergence, anthesis and ripening) of wheat are controlled by vernalization and photoperiod response, and earliness *per se* genes (Kosner & Pankova, 1998). These genes, along with their interaction with growth temperatures (Gororo et al., 2001), play a significant role in wheat's adaptation and yield potential in many environments. Vernalization response, or high temperature inhibition of reproductive development, is widespread in temperate plant species (Flood & Halloran, 1986). Winter wheat requires exposure to a continuous cold treatment (vernalization) prior to reproductive initiation. Spring wheat generally does not have such a requirement, but some cultivars do respond to cold treatment by flowering early (Levy & Peterson, 1972; Jedel et al., 1986). Vernalization and photoperiod sensitivity play key roles in adaptation, as they act to protect delicate floral primordia from extreme temperatures by accelerating or delaying ear initiation (Law & Worland, 1997).

Vernalization and photoperiod affect floral initiation time, leaf number, timing of other growth stages up to emergence of the flag leaf, tiller number (Levy & Peterson,

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1972) and spikelet number (Gororo et al., 2001; Whitechurch & Snape, 2003) in sensitive genotypes. Photoperiod and vernalization genes determine flowering in response to specific day length and temperature (Worland, 1996). A third genetic mechanism, deemed earliness *per se*, affects heading time independent of other genetic mechanisms. Earliness *per se* is the difference in flowering or maturation of plants whose vernalization and photoperiod requirements have been fulfilled (Kato et al., 2001). Under field conditions, photoperiod response and earliness *per se* affect flowering time in both spring and winter wheat (Kato & Yamashita, 1991). Vernalization affects flowering time in spring wheat as this requirement is generally not fulfilled during the growing season (Ortiz-Ferrara et al., 1995). The photoperiod requirement of spring wheat in high northern latitudes is generally fulfilled during the growing season (Worland et al., 1998; Marshall et al., 1989) and flowering time is, therefore, mainly affected by vernalization and/or earliness *per se* genes.

The specific genes governing the range of maturity in Canadian spring wheat cultivars are presently not known. Cold temperatures at planting and at early growth stages may alter vernalization pathways in responsive spring wheat genotypes. Moreover, the relationship of *Vrn* alleles to other important agronomic traits in western Canada needs to be investigated (Jedel, 1994). Due to inconsistent vernalization conditions in western Canada, vernalization responsive spring wheat genotypes tend to exhibit variable days to maturity and yield potential (Jedel, 1994).

A better understanding of the underlying genetic control of agronomically important traits in relation to vernalization response may aid in breeding for early maturity. The present study was conducted to, 1) determine which of the three genetic factors (vernalization, photoperiod, and earliness *per se*) cause flowering/maturity differences in western Canadian adapted spring wheat cultivars, and 2) investigate the relative importance of GCA and SCA effects in controlling earliness of flowering/maturity, and other agronomic traits under different vernalization treatments in the F_1 hybrids obtained from a one-way diallel cross among five cultivars.

2.2 Materials and Methods

Plant Material: Five Canadian spring wheat cultivars spanning the range in maturity of western Canadian adapted hexaploid spring wheat were selected for this study. The cultivars included 'AC Taber (late)' (Knox et al., 1992), 'AC Foremost (medium)' (Thomas et al., 1997), 'AC Barrie' (medium) (McCaig et al., 1995), 'AC Intrepid' (early) (DePauw et al., 1999) and 'Cutler' (early) (Briggs et al., 1991). The respective days to maturity of these cultivars are 112, 109, 109, 103 and 102 days (Alberta Agriculture, 2002).

Experiment 1: The cultivars described above were vernalized for six weeks as sprouted seeds at 1°C in the dark. To provide control plants at the same growth stage, unvernalized seeds were germinated a week before the end of the vernalization treatment at room temperature. Following vernalization, the seedlings were kept at an intermediate temperature of 13°C for two days to prevent possible devernalization (Chujo, 1970). Four seedlings of each (vernalized and un-vernalized) cultivar were then transplanted into 15 cm diameter pots (thinned to two plants pot^{-1}) containing Metromix (W. R. Grace & Co., Ajax, Ontario, Canada), and were arranged in two growth chambers providing 16 hours and 10 hours of photoperiods through a combination of fluorescent tubes and incandescent lamps (light intensity of about 300 $\mu mol\ m^{-2}\ s^{-1}$ at plant level). Both growth chambers were maintained at 23°C and 18°C temperatures, each for 12 hours. The experimental design within each photoperiod treatment was a factorial combination of vernalization treatments and cultivars arranged as a randomized complete block (RCB) with five replications (each having 2 plants per treatment). The experiment was repeated twice (two blocks in time).

Plants were watered when the surface of the pot was dry and fertilized weekly with a water soluble commercial fertilizer (20-20-20: $N\text{-}P_2O_5\text{-}K_2O$). Observations were made on the main culm for final leaf number (FLN), number of days from transplanting to anthesis, number of spikelets $spike^{-1}$, plant height and number of tillers $plant^{-1}$. Data were analyzed in the MIXED procedure of SAS (SAS Institute, 1999). Photoperiod, vernalization and cultivar were considered fixed effects, while block, replication and

replication (photoperiod) were considered random. Heterogeneity of error variances between the block and photoperiod was allowed using the GROUP option in the REPEATED statement (Yang, 2002). An initial value of unity was specified for all the covariance parameters by the PARMS statement. Treatments were compared using ESTIMATE statements. Photoperiod response was considered as the difference between vernalized short and vernalized long photoperiod treatments. Vernalization response was measured as the difference of non-vernalized long and vernalized long photoperiod. Earliness *per se* was considered as the difference in days to anthesis or maturity among vernalized cultivars grown in long photoperiod (Gororo et al., 2001).

Experiment 2: The five cultivars were crossed in a one-way diallel mating design to obtain a total of 10 [(5(5-1)/2] cross combinations. One set of the ten F_1 hybrids and five parents (30 seeds per genotype) were vernalized for six weeks as sprouted seeds at 1°C in the dark as described previously. To provide control plants at the same growth stage, unvernalized seeds (30 per genotype) were germinated a week before the end of the vernalization treatment at room temperature. Two seedlings for each set (vernalized and un-vernalized) were then transplanted into 12.5 cm diameter pots (thinned to one plant pot^{-1}) containing Metromix. The transplants were arranged as a factorial combination of vernalization and genotype treatments (2×15) in a RCB with five blocks (each having two pots per treatment; a total of 10 plants per treatment) in a walk-in chamber maintained at 16 hours photoperiod and a 21±2°C constant temperature. A light intensity of about 300 µmol m^{-2} s^{-1} at plant level was supplied through a combination of fluorescent tubes and incandescent lamps. The plants were watered and fertilized as described previously. Observations were made on the main culm for final leaf number, number of days from transplanting to anthesis and maturity, and number of spikelets, and on number of tillers and grain yield $plant^{-1}$. Vernalization response and earliness *per se* was measured as described previously.

To test the significance of vernalization and genotypic effects, four sets of analyses were conducted in the MIXED procedure of SAS. For the purposes of estimating genotypic performances under vernalized and un-vernalized conditions, best

linear unbiased predictors (BLUPs) were obtained. The genotype effect was further partitioned into parents and hybrids (using BY GENOTYPE statement) and parents versus hybrids (using the ESTIMATE statement). Likelihood ratio testing was used to test if individual variance components were zero. Likelihood ratios were constructed as the differences between the -2 Residual Log Likelihood values of the reduced covariance model (without the effect being tested) and the full covariance model (with the effect being tested) (Yang, 2002). Likelihood ratio test probabilities were halved prior to comparing with Chi-square tabulated value (Self & Liang, 1987). Combining ability analyses were performed on mean plot values according to Griffing's method IV model II (Griffing, 1956), excluding parents and treating genotypes as random, separately for un-vernalized and vernalized treatments, and for vernalization response of the genotypes. Combining abilities and components of variance were estimated using Diallel Analysis and Simulation Software (Burow & Coors, 1994). To remove bias in combining ability estimates, parents were excluded from the diallel analyses (Das & Griffey, 1994; Singh et al., 1992). The significance of GCA and SCA effects was tested as deviations from zero using the *t-test*. Where SCA was significant, the ratio of the combining ability variance components $[(2\sigma^2_{GCA})/(2\sigma^2_{GCA}+\sigma^2_{SCA})]$ was calculated to determine the predictability of single-cross progeny performance from GCA (Baker, 1978). Assuming no epistatic effects, narrow-sense heritability was calculated as the ratio of additive variance to total phenotypic variance, $(2\sigma^2_{GCA})/(2\sigma^2_{GCA}+\sigma^2_{SCA}+\sigma^2_e)$ with σ^2_e being the residual variance (Falconer & Mackay, 1996).

2.3 Results

Experiment 1: The main effect of photoperiod was significant (*P<0.01*) for anthesis but not for leaf, tiller and spikelet number, and main culm height (Table 2.1). Vernalization altered (*P<0.01*) both leaf number and days to anthesis, but not the number of tillers and spikelets or main culm height. Photoperiod × vernalization interaction was significant for all traits except number of tillers plant^{-1}. Photoperiod × cultivar interaction was significant for leaf number and main culm height. Vernalization × cultivar interaction was significant for leaf and spikelet number, and for days to anthesis. Significant three-

way interactions of photoperiod, vernalization and cultivar were observed for days to anthesis and spikelet number.

A short photoperiod delayed anthesis in all cultivars, with 'Barrie' exhibiting the greatest response (Table 2.2). Shorter photoperiod also altered leaf number in 'Barrie' and main culm height in 'Intrepid' (data not shown). 'Taber' and 'Foremost' responded to vernalization, altering final leaf number, number of spikelets and days to anthesis. The number of spikelets in 'Taber' and 'Foremost' decreased when vernalized (Table 2.2). 'Barrie', 'Intrepid' and 'Cutler' did not respond to vernalization for any of the traits studied. When the cultivars were vernalized and grown under long photoperiods, only 'Barrie' differed from rest of the cultivars for days to anthesis.

Experiment 2: Combined analysis of variance revealed significant ($P<0.05$) variation due to vernalization and vernalization × genotype interaction (Table 2.3a). Genotypic variance was not significant ($P\geq0.05$) for any of the traits studied. Among the three components of genotypic variance (parents (P), hybrids (H) and P vs. H) only the latter was significant for days to maturity. Vernalization × genotype interaction variance was significant ($P<0.01$) for all traits, whereas that of vernalization × parent was not significant for number of tillers only. Vernalization × hybrids effect varied ($P<0.05$) for all traits except number of tillers and days to maturity. Best Linear Unbiased Predictor (BLUP) values of the parents and F_1 hybrids for various traits under un-vernalized and vernalized treatments are presented in Table 2.4.

Significant ($P<0.01$) genotypic variation existed for all traits studied when not vernalized (Table 2.3b). Among genotypic components, variances due to parents and hybrids were significant ($P<0.05$) for all traits except number of tillers. Estimates of parents vs. hybrids were significant ($P<0.01$) for final leaf number and days to anthesis, but not for other traits studied. The GCA effect when not vernalized was significant ($P<0.05$) for all traits except tillers plant^{-1}, while the SCA effect was significant ($P<0.05$) for grain yield only. Correlations between parental BLUPs and GCA effects were high for FLN (r=0.93; $P<0.05$), days to anthesis (r=0.98; $P<0.01$) and maturity (r=0.98;

P<0.01), and spikelet number (r=0.95; *P<0.01*). Narrow-sense heritability was low for tiller number (0.20) but high for FLN (0.83), days to anthesis (0.92) and maturity (0.71), spikelet number (0.86) and yield (0.62) under un-vernalized treatment.

Genotypes varied (*P<0.05*) for all traits when vernalized (Table 2.3c). Parents varied for days to anthesis and spikelet number only. Hybrids did not differ (*P≥0.05*) for final leaf number and days to maturity. The estimate of parents vs. hybrids was significant for all traits (*P<0.01*). The GCA effect was significant (*P<0.01*) for days to anthesis, number of tillers and spikelets and yield, while SCA was not significant. Estimates of narrow-sense heritability for FLN (0.10), days to anthesis (0.63) and maturity (0.24), number of tillers (0.57) and spikelets (0.77), and yield (0.62) under vernalized treatment were lower than those for un-vernalized treatment and vernalization response.

Genotypes varied (*P<0.01*) for all traits except tiller number for vernalization response (Table 2.3d). Parents differed for all traits but tiller number and days to maturity. The estimates of parents vs. hybrids were significant (*P<0.05*) for final leaf number, days to anthesis, spikelet number and yield, but not for days to maturity and tiller number. The F_1 hybrids also differed for all traits except tiller number and days to maturity. The GCA effects were significant (*P<0.05*) for all traits, while SCA effects were not. High correlations between parental BLUPs and GCA effects for FLN (r=0.96; *P<0.01*), days to anthesis (r=0.98; *P<0.01*) and maturity (r=0.94; *P<0.05*), spikelet number (r=0.97; *P<0.01*) and grain yield (r=-0.93; *P<0.05*) were observed for vernalization response. Narrow-sense heritability under vernalization response was low for tiller number (0.25) and days to maturity (0.32), medium for yield (0.53) and relatively high for final leaf number (0.80), spikelet number (0.86) and days to anthesis (0.93).

Positive GCA or SCA values reflect the combining ability for high leaf and spikelet number and yield, and for late anthesis and maturity, while negative values connate the converse. 'Taber' exhibited the greatest positive GCA effect for leaf and

spikelet number, days to anthesis for vernalization response and when vernalized; for tiller number under vernalization response, and for days to maturity when either vernalized or not (Table 2.5). 'Barrie' had the highest significant positive GCA effects for tiller number and grain yield when either vernalized or not, and for days to anthesis and spikelet number when vernalized. 'Intrepid' exhibited the greatest negative GCA effect for leaf and tiller number and days to anthesis for vernalization response and when not vernalized; for grain yield when either vernalized or not, and for days to maturity and spikelet number when not vernalized (Table 2.5). 'Foremost' exhibited the highest significant negative GCA effect for number of spikelets and tillers and days to anthesis when vernalized. 'Taber' had the highest significant negative GCA for yield while 'Barrie' that for spikelet number for vernalization response.

The hybrid 'Barrie × Intrepid' had the highest negative SCA (-1.37, -1.43; *P<0.05*) for tiller number under vernalization response and un-vernalized treatment, respectively. The hybrid 'Taber × Foremost' exhibited positive SCA effect (0.83, *P<0.05*) for days to anthesis when vernalized, while 'Foremost × Intrepid' showed negative SCA effects (-1.3 and -1.2; *P<0.05*) for the same trait for vernalization response and un-vernalized treatment, respectively. The only significant SCA effect (-2.37, *P<0.05*) for days to maturity was observed for 'Taber × Barrie' when not vernalized. In the absence of vernalization, the hybrids 'Cutler × Foremost', 'Cutler × Intrepid', and 'Foremost × Barrie' exhibited significant SCA effects (-1.65, *P<0.05*; 1.75, *P<0.01*; and 1.63, *P<0.05*, respectively) for grain yield. Significance of SCA suggests the involvement of non-additive gene effects in these hybrids. All the F_1 hybrids of 'Taber', 'Foremost × Cutler' and 'Foremost × Barrie' deviated (*P<0.05*) from mid-parents for days to anthesis for vernalization response (data not shown) and un-vernalized treatment (Figure 2.4), further suggesting the presence of non-additive gene action. Most of these hybrids exhibited complete dominance and / or some over-dominance from their parents. All F_1 hybrids of 'Taber' deviated from mid-parents and showed either over or complete dominance for spikelet number when vernalized (Figure 2.5). The F_1 hybrids 'Taber × Foremost' and 'Cutler × Foremost' deviated from mid-parents for final leaf number under vernalization response and un-vernalized treatment, respectively.

2.4 Discussion and Conclusions

When vernalization and photoperiod requirements were fulfilled, 'Taber', 'Foremost', 'Intrepid' and 'Cutler' flowered at the same time. However, under field conditions 'Taber' and 'Foremost' flower later (Alberta Agriculture, 2002) because their vernalization requirement is probably not fulfilled. The present study suggests that flowering time of 'Foremost' and 'Taber' is mainly affected by vernalization response, while 'Barrie', 'Intrepid' and Cutler are influenced mainly by earliness *per se* genes.

'Taber' and 'Foremost' belong to the Canada prairie spring wheat class and are developed from crosses with CIMMYT wheat germplasm. The higher yield potential of these cultivars (in high northern growing regions) is related in part to their relative late maturity as a result of their vernalization responsiveness. Vernalization response genes are known to contribute indirectly to yield by influencing flowering time (Flood & Halloran, 1986), tiller (Levy & Peterson, 1972) and spikelet number (Gororo et al., 2001; Whitechurch & Snape, 2003) in sensitive genotypes. Both 'Taber' and 'Foremost' have most probably inherited their vernalization responsiveness from their common parent 'HY320' (Cutforth et al., 1992). This vernalization response may thus confer yield advantages in northern growing regions, but may sometimes be associated with variable and late maturity.

The five cultivars and their F_1 hybrids exhibited differential response to vernalization for all traits. Following vernalization, final leaf number, number of tillers and spikelets, days to anthesis and grain yield decreased in 'Taber' and 'Foremost', and hybrids involving them. Based on these results, 'Taber' and 'Foremost' appear to carry a different spring habit allele from rest of the cultivars studied, at major loci governing vernalization response. This allele conferred early anthesis and maturity when vernalized, and late anthesis and maturity when not vernalized. Differential responses to vernalization of Canadian spring wheats have been reported by Major and Whelan (1985) and Jedel et al. (1986), with responsive cultivars in those studies flowering earlier when vernalized. Hucl and Baker (1987) reported that tillering differences across years for the cultivar 'HY320' may be due to variable vernalization temperatures. Such variable

vernalizing temperatures may result in inconsistent maturity and yield patterns in responsive genotypes, and must be considered in breeding programs (Jedel, 1994).

In the absence of vernalization, flowering and maturity time are influenced by vernalization response and / or earliness *per se* genes. When not vernalized, additive gene effects were found to be more important than non-additive effects in all traits studied. Selection for early flowering/maturity in early generations under field conditions can, therefore, result in genetic improvement in earliness.

When vernalized, the greater importance of GCA and the non-significance of SCA for days to anthesis, number of tillers and spikelets and yield, and the high components of variance ratio for leaf number and days to maturity indicate that additive gene action is more important for these traits. As both the photoperiod and vernalization requirements were fulfilled, the resulting variation is attributable to earliness *per se*. This suggests that the action of earliness *per se* genes is also mainly additive in western Canadian adapted spring wheat cultivars.

The difference between un-vernalized and vernalized treatments depicts the effect of vernalization response genes. The significance of GCA and non-significance of SCA suggest that additive gene action is more important than non-additive in determining vernalization response in western Canadian adapted spring wheats. These results suggest that the negative impact of vernalization response in western Canadian adapted spring wheats could be avoided to some extent by using early maturing cultivars as one of the breeding parents. Final leaf number, days to anthesis and spikelet number were affected the most by vernalization. These traits had high heritabilities and thus selection for these traits in early generations could eliminate vernalization response.

The significance of parents vs. hybrids suggests the presence of non-additive genetic effects for all traits when vernalized; final leaf number and days to anthesis when not vernalized; and final leaf and spikelet number, days to anthesis and yield in vernalization response. When vernalized, only 'Taber × Foremost' deviated from the

mid-parent value for days to anthesis. This hybrid also exhibited significant SCA, suggesting the presence of non-additive effects of earliness *per se* genes. Kato and Wada (1999) reported earliness *per se* to be partially dominant over lateness in four hybrids of spring wheat. In the present study, five of ten hybrids exhibited complete dominance of earliness and lateness *per se* for days to anthesis. All F_1 hybrids of 'Taber', 'Foremost × Cutler' and 'Foremost × Barrie' deviated from mid-parents for days to anthesis in un-vernalized plants and vernalization response. These hybrids exhibited complete dominance in the direction of 'Taber' or 'Foremost', suggesting the non-additive effects of vernalization response genes for days to anthesis. All hybrids of 'Taber' deviated from mid-parent values for spikelet number when vernalized and exhibited dominance for high spikelet number, suggesting some non-additive response when cold treated. Rahman et al. (1978) also reported dominance for high spikelet number, but not F_1-midparent deviation. The F_1-midparent and/or dominance deviations, in our study, did not explain all of the significant SCA effects for various traits.

The estimates of narrow-sense heritability for final leaf number, days to anthesis and maturity, and spikelet number when vernalized were relatively lower than when not vernalized and for vernalization response. Dahanayake and Galwey (1999) reported higher heritabilities in the absence of vernalization compared to vernalization response for number of leaf nodes at flowering and time to flowering in spring rape (*B. napus*). Heritability estimates for grain yield are consistent with those obtained under field conditions by Hill et al. (1999), May and van Sanford (1992) and Anwar and Chowdhry (1969). Heritability for days to maturity in un-vernalized plants was similar to those reported by May and van Sanford (1992). However, heritability estimates are higher than those of May and van Sanford (1992) and Nanda et al. (1981) for days to anthesis, and those of Nanda et al. (1981) and Kronstad and Foote (1964) for spikelet number.

Under short-season western Canadian growing conditions, vernalization non-responsiveness is generally considered a preferred phenotype (Jedel, 1994) to avoid inconsistent maturity and yield patterns. However, for developing widely adapted wheat cultivars, the use of vernalization responsive genes in combination with non-responsive

genes has been recommended (Stelmakh, 1993; Jedel, 1994). Results of this study suggest that additive gene action plays a major role in the inheritance of both vernalization response and earliness *per se*. The relative importance of additive over non-additive gene effects suggests that crossing genotypes with the highest negative GCA for days to maturity would result in the earliest maturing progeny. High GCA for vernalization response in western Canadian adapted spring wheats indicates that genetic gain can be expected from selection against or in favor of vernalization response. High narrow-sense heritability of final leaf number, days to anthesis and spikelet number for vernalization response and un-vernalized treatment suggests that selection for the minimum difference between these traits or for the same traits under non-vernalizing temperatures would help eliminate vernalization response in early stages of a breeding program. Initial selection against major vernalization response genes could result in early flowering and maturity at the outset of a selection program. Following the elimination of vernalization response, selection for earliness *per se* genes could fine-tune flowering and maturity time for selected environments.

2.5 Summary

Under short-season western Canadian growing conditions, vernalization non-responsiveness is generally considered a preferable spring wheat (*Triticum aestivum* L.) phenotype, to avoid inconsistent maturity and yield patterns. The genetic factors affecting early flowering, maturity, and related agronomic traits, were investigated in a set of five Canadian spring wheat cultivars. The cultivars were first studied under 10 and 16 hour photoperiods and 0 and 42 day vernalization treatments. Thereafter, the parents and F_1 hybrids from a one-way diallel mating design were grown with and without a 42 day vernalization treatment. Shorter photoperiod delayed flowering time in all cultivars, and increased final leaf number in 'AC Barrie'. Vernalization hastened flowering and decreased final leaf number in 'AC Foremost' and 'AC Taber'. 'AC Foremost' and 'AC Taber' carry a different spring habit allele from the rest of the cultivars studied, at the major loci governing vernalization response. Leaf and spikelet number on the main culm, days to anthesis and maturity, tiller number and yield plant^{-1} were mainly controlled by additive gene action. Narrow-sense heritability was medium to high (0.53-0.93) for final leaf number, days to anthesis, spikelet number and grain yield, but low to medium (0.20-

0.71) for days to maturity and tiller number. Selection for early flowering under non vernalizing conditions may aid in the breeding of (vernalization non-responsive) early maturing spring wheat cultivars in western Canada.

2.6 Tables and Figures

Table 2.1. Analysis of variance for testing the effect of photoperiod and vernalization on days to anthesis and other agronomic traits of five spring wheat cultivars.

Source	Significance of Fixed Effects (Pr>F)				
	Final leaf number	Tiller number	Days to Anthesis	Spikelet number	Plant height
Photoperiod (P)	0.301	0.97	<.0001	0.19	0.18
Vernalization (V)	0.0003	0.63	<.0001	0.17	0.15
P × V	0.049	0.79	0.007	<.0001	0.004
Cultivar (C)	<.0001	0.12	0.0001	0.25	0.25
C × P	0.006	0.32	0.15	0.34	0.02
C × V	0.003	0.46	0.002	0.03	0.35
C × P × V	0.15	0.57	0.035	0.0002	0.31

Table 2.2. Photoperiod and vernalization responses of five Canadian spring wheat cultivars differing in time to maturity (please see materials & methods for definitions).

Cultivar	Trait											
	Final leaf number			Tiller number			Days to Anthesis			Spikelet number		
a) Photoperiod Response (ΔP)												
	VS[y]	VL[y]	ΔP	VS	VL	ΔP	VS	VL	ΔP	VS	VL	ΔP
Taber	9.2	8.9	*ns*	5.1	5.1	*ns*	58	43	15**	15	15	*ns*
Cutler	9.6	8.7	*ns*	5.5	5.2	*ns*	58	41	17**	20	18	*ns*
Foremost	8.5	8.5	*ns*	4.5	4.5	*ns*	52	39	13**	16	16	*ns*
Barrie	12.5	9.2	3.3*	10.4	9.5	*ns*	>100	49	>50**	-	18	-
Intrepid	8.1	8.1	*ns*	5.8	5.8	*ns*	51	39	12**	16	16	*ns*
$SE_{difference}$	**0.3**	**0.2**		**0.6**	**0.8**		**1.4**	**0.9**		**0.7**	**0.5**	
b) Vernalization Response (ΔV)												
	0L[x]	VL	ΔV	0L	VL	ΔV	0L	VL	ΔV	0L	VL	ΔV
Taber	11.1	8.9	2.2**	5.9	5.1	*ns*	57	43	14**	20	16	4*
Cutler	8.7	8.7	*ns*	5.9	5.2	*ns*	43	41	*ns*	18	18	*ns*
Foremost	10	8.6	1**	5.5	4.5	*ns*	50	39	11**	18	15	3*
Barrie	9.6	9.2	*ns*	9.6	9.5	*ns*	51	49	*ns*	18	18	*ns*
Intrepid	8.4	8.1	*ns*	6.3	5.8	*ns*	40	39	*ns*	16	16	*ns*
$SE_{difference}$	**0.2**	**0.2**		**0.8**	**0.8**		**0.9**	**0.9**		**0.5**	**0.5**	

[y]VS= Vernalized short-day; VL= Vernalized long-day.
[x]0L= Non-vernalized long-day.
**,* Significant at *P<0.01* and *P<0.05*, respectively.
ns Not significant. - Non-estimable (No flowering).

Table 2.3. Analyses of variance for various agronomic traits as affected by vernalization in a one-way diallel cross among five spring wheat cultivars differing in maturity.

Source	Likelihood Ratio Test Statistics					
	Final leaf number	Tillers	Anthesis	Maturity	Spikelets	Yield
a) Combined						
Block	3.4	0.1	1.0	0.6	1.0	9.9**
Vernalization (V)[z]	-***	-***	-***	-***	-***	-***
Genotype (G)	0	0.4	0	2.6	0	0.5
Parents (P)	0.1	0.2	0	0.2	0.1	0.2
Hybrids (H)	0	0.2	0	2.0	0	0.4
P vs H[y]	0	-1.9	0.4	24*	0	-3.1
V × G	33**	4.4*	81**	5.5*	59**	23**
V × P	8.1**	1.7	32**	5.5*	28*	9.7**
V × H	18**	2.1	47**	1.4	30**	11**
b) Un-vernalized						
Block	0.4	0	1.7	0.6	0.4	4.1*
Genotype	33**	4.3*	58**	23**	40**	25**
Parents	14**	3.3	23**	8.1**	20**	12**
P vs H[y]	4.9**	-0.1	41**	28	-0.2	0.2
Hybrids	20**	2.2*	33**	13**	20**	13**
GCA[x]	0.7*	0.4	16.3*	9.6*	2.3*	4.4*
SCA[x]	0.02	0.6	0.3	0.4	-0.1	1.3*
c) Vernalized						
Block	1.1	3.1	0	0	0.4	4.6*
Genotype	3.2	12**	27**	4.0*	33**	15**
Parents	0.1	1.7	12**	0.7	18**	3.1
P vs H[y]	-3.1**	-8.7**	12**	24**	-14**	-13**
Hybrids	0.6	8.5**	12**	0.3	13**	7.7**
GCA	0.01	0.6**	0.99*	1.3	0.8*	1.05*
SCA	0.01	0.06	0.3	-0.3	-0.1	-0.06
d) Vernalization Response (difference b/w un-vernalized and vernalized)						
Block	0	0.1	2.5	0	0	0.1
Genotype	23**	3.1	59**	4.3*	42**	15**
Parents	5.3*	1.1	26**	3.6*	26**	7.5**
P vs H[y]	9.1**	5.8	28**	-3.9	13**	13*
Hybrids	14**	2.0	34**	1.1	21**	7.1**
GCA	0.70*	0.6	19*	2.7*	3.8*	3.4*
SCA	-0.1	0.4	0.3	-0.5	-0.1	1.03

***,**,* Significant at *P<0.001*, *P<0.01*, and *P<0.05* respectively.
No asterisk indicates non-significant effect (*P≥0.05*);
[z]Fixed effect.
[y]Estimates of parents versus hybrids.
[x]GCA, SCA = General and Specific Combining Ability, respectively (columns against GCA and SCA contain variance components).

Table 2.4. Best Linear Unbiased Predictors (BLUPs) of genotypes for various agronomic traits under un-vernalized (UV) and vernalized (V) treatments in a one-way diallel cross among five spring wheat cultivars differing in maturity.

Genotype	Final leaf number		Tillers plant^{-1} (no)		Anthesis (days)		Maturity (days)		Spikelets spike^{-1} (no)		Yield plant^{-1} (gm)	
Parents	UV	V	UV	V	UV	V	UV	V	UV	V	UV	V
Taber	9.7	7.4	10	5.7	50	36	94	84	22	17	15.5	6.5
Cutler	7.5	7.2	8.9	7.0	38	37	84	83	17	16	10.3	6.6
Foremost	9.0	7.0	8.6	4.3	44	34	92	84	20	14	11.9	4.1
Barrie	8.2	7.2	10.1	6.7	42	39	84	84	19	17	14.2	8.0
Intrepid	7.7	7.0	8.0	6.0	39	37	83	82	15	15	7.0	5.9
Hybrids												
Tab × Cut	9.4	6.9	8.9	4.9	50	38	93	88	20	14	12.8	5.3
Tab × For	10.1	6.6	9.6	4.1	55	37	97	86	22	13	14.0	3.3
Tab × Bar	9.9	7.1	10.4	5.1	51	39	89	86	20	15	14.0	5.6
Tab × Int	9.0	6.9	8.2	4.9	48	38	91	87	18	14	8.6	4.2
Cut × For	9.4	7.0	8.8	4.7	46	36	92	87	19	14	11.6	4.9
Cut × Bar	8.2	7.0	10.2	6.4	43	39	87	85	18	16	13.0	6.7
Cut × Int	7.5	6.9	9.2	5.9	40	38	85	85	16	14	9.8	4.6
For × Bar	9.6	6.9	9.8	5.2	49	37	91	86	20	14	16.2	5.9
For × Int	8.6	6.6	8.2	3.8	44	37	89	85	18	13	9.4	3.4
Bar × Int	7.5	6.7	7.7	5.9	43	40	86	84	17	15	8.7	5.4
SE[y]	**0.32**	**0.23**	**0.86**	**0.65**	**0.98**	**0.60**	**1.8**	**1.6**	**0.58**	**0.44**	**1.1**	**0.75**

[y] Standard error of the difference between BLUPs within column.

Table 2.5. General combining ability estimates of various agronomic traits for vernalization response (upper), un-vernalized (middle) and vernalized (lower) treatment in a one-way diallel cross among five spring wheat cultivars differing in maturity.

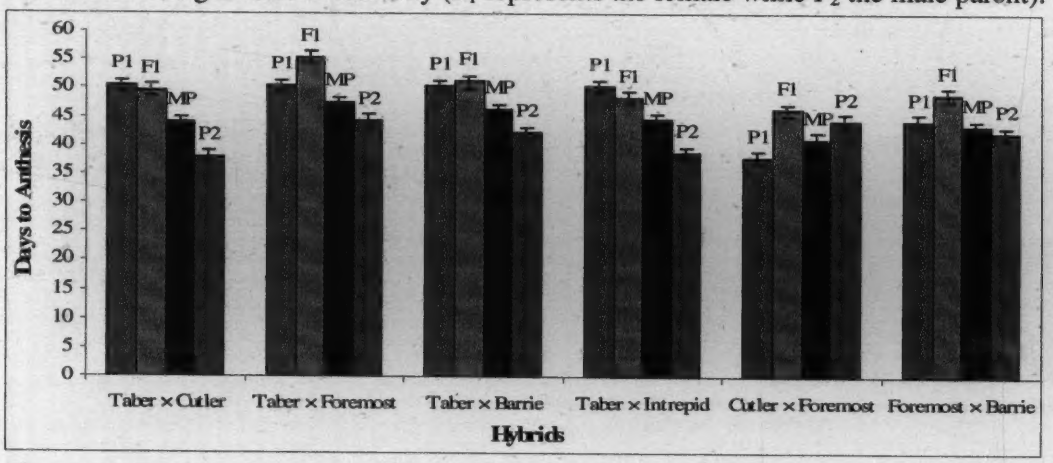
Traits	Parents				
	Taber	Cutler	Foremost	Barrie	Intrepid
	0.92**	-0.61**	0.85**	*ns*	-0.88**
Final Leaf Number	0.97**	-0.43**	0.71**	*ns*	-1.09**
	ns	*ns*	*ns*	*ns*	*ns*
	1.0*	*ns*	*ns*	*ns*	-1.32*
Tillers plant^{-1} (no)	*ns*	*ns*	*ns*	0.87*	-1.53*
	-0.56**	0.64**	-1.03**	0.91**	*ns*
	5.56**	-2.64**	3.69**	-2.04**	-4.57**
Anthesis (days)	5.69**	-3.24**	2.29*	*ns*	-4.17**
	ns	*ns*	-1.55**	1.32**	*ns*
	1.99*	*ns*	2.12*	*ns*	*ns*
Maturity (days)	3.85**	*ns*	3.12**	-2.35**	-3.41**
	1.80*	*ns*	*ns*	*ns*	*ns*
	2.19**	-0.95**	2.05**	-1.75**	-1.55**
Spikelets spike^{-1} (no)	1.75**	*ns*	1.21**	*ns*	-2.12**
	ns	0.60**	-1.13**	1.20**	*ns*
	-3.38**	*ns*	*ns*	*ns*	2.61*
Yield plant^{-1} (gm)	*ns*	*ns*	1.44**	1.66**	-3.88**
	-0.54*	0.70*	-0.83**	1.51**	-0.84**

**,* Significant at $P<0.01$ and $P<0.05$, respectively;
ns Not significant ($P\geq0.05$).

Figure 2.1. Sprouted seeds being vernalized

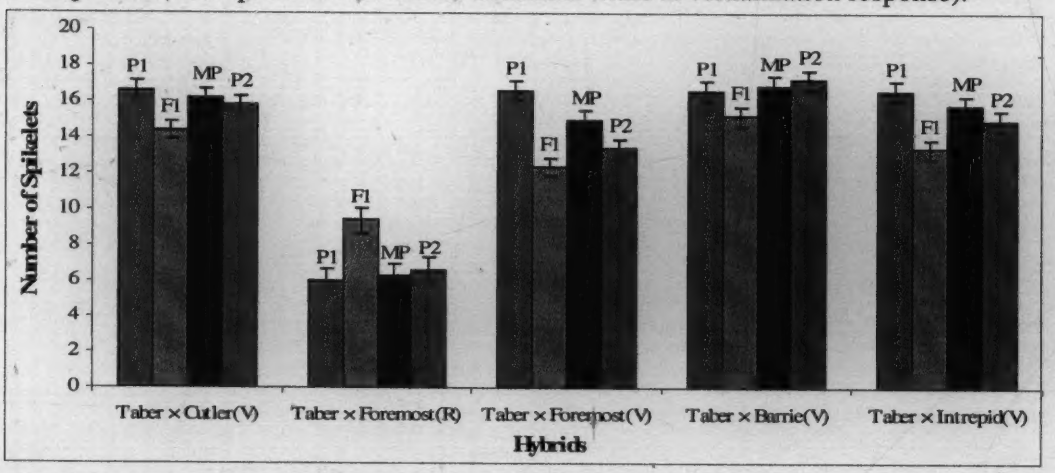
Figure 2.2. Differences in flowering time and spikelet number of Unvernalized (left) and vernalized (right) Taber.

Figure 2.3. Deviations of F_1s from their mid-parental values (MP) for days to anthesis under un-vernalized condition in a one-way diallel cross among five spring wheat cultivars differing in time to maturity (P_1 represents the female while P_2 the male parent).



Error bars indicate the standard error of the difference between means in both directions.

Figure 2.4. Deviations of F_1s from their mid-parental values (MP) for spikelet number in a one-way diallel cross among five spring wheat cultivars differing in time to maturity (V in the parentheses represents vernalized treatment while R vernalization response).



Error bars indicate the standard error of the difference between means in both directions.

2.7 References

Alberta Agriculture. 2002. Varieties of cereal and oilseed crops for Alberta.

Anonymous. 1987. HY 320 red spring wheat. Can. J. Plant Sci. 67: 807-811.

Anwar, A.R., and A.R. Chowdhry. 1969. Heritability and inheritance of plant height, heading date and grain yield in four spring wheat crosses. Crop Sci. 9: 760-761.

Baker, R. J. 1978. Issues in diallel analysis. Crop Sci. 18: 533-536.

Briggs, K. G., S. Kibite, and K. Kutschera. 1991. Cutler red spring wheat. Can. J. Plant Sci. 72: 229-233.

Burow, M. D., and J.G. Coors. 1994. DIALLEL: A microcomputer program for the simulation and analysis of Diallel crosses. Agron. J. 86: 154-158.

Chujo, H. 1970. Some observations on the reversal of vernalization effect of wheat. Jpn. J. Crop Sci. 23: 1203-1205.

Cutforth, H. W., Y.W. Jame, and P.G. Jefferson. 1992. Effect of temperature, vernalization and water stress on phyllochron and final main-stem leaf number of HY320 and Neepawa spring wheats. Can. J. Plant Sci. 72: 1141-1151.

Dahanayake, S. R., and N.W. Galwey. 1999. Diallel analysis of vernalization responses in spring rape (*Brassica napus* L.): a basis for adaptation to a Mediterranean environment. Aus. J. Agric. Res. 50: 1417-1423.

Das, M. K., and C.A. Griffey. 1994. Diallel analysis of adult plant resistance to powdery mildew in wheat. Crop Sci. 34, 948-952.

DePauw, R. M., J.M. Clarke, R.E. Knox, M.R. Fernandez, T.N. McCaig, and J.G. McLeod. 1999. AC Intrepid hard red spring wheat. Can. J. Plant Sci. 79: 375-378.

Falconer, D. S., and T.F.C. Mackay. 1996. Introduction to quantitative genetics. Prentice Hall, Harlow, England.

Flood, R. G., and G.M. Halloran. 1986. Genetics and physiology of vernalization response in wheat. Adv. Agron. 39: 87-124.

Gardner, C. O., and S.A. Eberhart. 1966. Analysis and interpretation of the variety cross diallel and related populations. Biometrics, 22: 439-452.

Gororo, N. N., R.G. Flood, R.F. Eastwood, and H.A. Eagles. 2001. Photoperiod and vernalization responses in *T. turgidum* × *T. tauschii* synthetic hexaploid wheats. Ann. Bot. 88: 947-952.

Griffing, B. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. Aus. J. Biol. Sci. 9: 463-493.

Hill, J., R. Ortiz, W.W. Wagoire, and O. Stolen. 1999. Effectiveness of indirect selection for wheat yield in a stress environment. Theor. Appl. Genet. 98: 305-309.

Hucl, P., and R.J. Baker. 1987. A study of ancestral and modern Canadian spring wheats. Can. J. Plant Sci. 67: 87-97.

Hucl, P., and M. Matus-Cadiz. 2002. CDC EMDR-4, CDC EMDR-9, and CDC EMDR-14 spring wheats. Can. J. Plant Sci. 82: 411-413.

Jedel, P.E. 1994. Inheritance of vernalization response in three populations of spring wheat. Can. J. Plant Sci. 74: 753-757.

Jedel, P.E., L.E. Evans, and R. Scarth. 1986. Vernalization responses of a selected group of spring wheat (*Triticum aestivum* L.) cultivars. Can. J. Plant Sci. 66: 1-9.

Kato, K., and T. Wada. 1999. Genetic analysis and selection experiment for narrow-sense earliness in wheat by using segregating hybrid progenies. Breed. Sci. 49: 233-238.

Kato, K., and S. Yamashita. 1991. Varietal variation in photoperiodic response, chilling requirement and narrow-sense earliness and their relation to heading time in wheat (*Triticum aestivum* L.). Japan. J. Breed. 41: 475-484.

Kato, K., S. Taketa, T. Ban, N Iriki, and K. Miura. 2001. The influence of a spring habit gene, *Vrn-D1*, on heading time in wheat. Plant Breed. 120: 115-120.

Knox, R. E., R.M. DePauw, R.J. Morrison, T.N. McCaig, J.M. Clarke, and J.G. McLeod. 1991. AC Taber red spring wheat. Can. J. Plant Sci. 72: 1241-1245.

Kosner, J., and K. Pankova. 1998. The detection of allelic variants at the recessive *vrn* loci of winter wheat. Euphytica, 101:9-16.

Kronstad, W. E., and W.H. Foote. 1964. General and specific combining ability estimates in winter wheat (*Triticum aestivum* L.). Crop Sci. 4: 616-619.

Law, C. N., and A.J. Worland. 1997. Genetic analysis of some flowering time and adaptive traits in wheat. New Phytologist, 137: 19-28.

Levy, J., and M.L. Peterson. 1972. Responses of spring wheats to vernalization and photoperiod. Crop Sci. 12: 487-490.

Major, D. J., and E.D.P. Whelan. 1985. Vernalization and photoperiod response characteristics of a reciprocal series of Rescue and Cadet hard red spring wheat. Can. J. Plant Sci. 65: 33-39.

Marshall, L., R. Busch, F. Cholick, I. Edwards, and R. Frohberg. 1989. Agronomic performance of spring wheat isolines differing for daylength response. Crop Sci. 29: 752-757.

May, L., and D.A. van Sanford. 1992. Selection for early heading and correlated response in maturity of soft red winter wheat. Crop Sci. 32: 47-51.

McCaig, T. N., R.M. DePauw, J.M. Clarke, J.G. McLeod, M.R. Fernandez, and R.E. Knox. 1995. AC Barrie hard red spring wheat. Can. J. Plant Sci. 76: 337-339.

Nanda, G.S., G.N. Hazarika, and K.S. Gill. 1981. Inheritance of heading date, plant height, ear length and spikelets per spike in an intervarietal cross of wheat. Theor. Appl. Genet. 60: 167-171.

Ortiz-Ferrara, G., M.G. Mossad, V. Mahalakshmi, and R.A. Fischer. 1995. Photoperiod and vernalization response of wheat under controlled and field conditions. Plant Breed. 114: 505-509.

Rahman, M. S,, G.M. Halloran, and J.H. Wilson. 1978. Genetic control of spikelet number per ear with particular reference to rate of spikelet initiation in hexaploid wheat (*Triticum aestivum* L.). Euphytica, 27: 69-74.

SAS Institute. 1999. SAS software. Version 8.1. SAS Institute, Inc. Cary, NC.

Self, S.G., and K-Y. Liang. 1987. Asymptotic properties of maximum likelihood estimators and likelihood ratio tests under nonstandard conditions. J. Am. Stat. Assoc. 82: 605-610.

Singh, O., C.L.L. Gowda, S.C. Sethi, T. Dasgupta, and J.B. Smithson. 1992. Genetic analysis of agronomic characters in chickpea. I. Estimates of genetic variances from diallel mating designs. Theor. Appl. Genet. 83: 956-962.

Stelmakh, A. F. 1993. Genetic effect of *Vrn* genes on heading date and agronomic traits in bread wheat. Euphytica, 65: 53-60.

Thomas, J. B., R.M. DePauw, R.E. Knox, E. Czarnecki, A.B. Campbell, J. Nielsen, R.I.H. McKenzie, K.J. Degenhardt, and R.J. Morrison. 1997. AC Foremost red spring wheat. Can. J. Plant Sci. 77: 657-660.

Whitechurch, E. M., and J.W. Snape. 2003. Developmental responses to vernalization in wheat deletion lines for chromosomes 5A and 5D. Plant Breed. 122: 35-39.

Worland, A.J. 1996. The influence of flowering time genes on environmental adaptability in European wheats. Euphytica, 89: 49-57.

Worland, A. J., A. Borner, V. Korzun, W.M. W.M. Li, S. Petrovic, and E.J. Sayers. 1998. The influence of photoperiod genes on the adaptability of European winter wheats. Euphytica, 100: 385-394.

Xiang, B., and B. Li. 2001. A new mixed analytical method for genetic analysis of diallel data. Can. J. For. Res. 31: 2252-2259.

Yang, R-C. 2002. Likelihood-based analysis of genotype-environment interactions. Crop Sci. 42: 1434-1440.

Chapter 3

Inheritance of Flowering and Maturity Time in Canadian Spring Wheat[3]

3.1 Introduction

Early maturity is one of the important objectives in spring wheat (*Triticum aestivum* L.) breeding programs globally. Earliness ensures timely crop harvest and may also protect wheat from biotic and abiotic stresses such as disease, heat and drought (Poehlman & Sleper, 1995). Due to the short growing season (95-125 days) in western Canada, the development of early maturing cultivars is important to avoid frost damage which can lower production and quality.

The genetic control of flowering/maturity time in wheat is complex, being determined by three component traits, photoperiod sensitivity, vernalization requirement and earliness *per se* (Kato and Yamagata, 1988). Photoperiod sensitivity is the requirement of a certain period of long day to initiate flowering. Vernalization requirement is the requirement of exposure to cold temperatures to initiate or accelerate flowering. Earliness *per se* is the difference in developmental rate, independent of day length and vernalization response. Genes of these three systems, along with their interaction with growth temperatures (Gororo et al., 2001), play a significant role in wheat's adaptation and yield potential in many environments. The photoperiod requirement of spring wheat in higher northern latitudes is generally fulfilled during the growing season (Marshall et al., 1989; Worland et al., 1998) and flowering time is, therefore, mainly affected by vernalization and/or earliness *per se* genes.

Vernalization sensitivity/insensitivity in hexaploid wheat is controlled by alleles at the major vernalization loci, *Vrn-A1*, *Vrn-B1*, *Vrn-D1* and *Vrn-D5* (Pugsley, 1972). Winter wheat possesses recessive alleles at all of these loci while spring wheat has dominant alleles at one or more of them. The dominant allele of *Vrn-A1* confers complete insensitivity to vernalization and is epistatic to the dominant alleles of *Vrn-B1*, *Vrn-D1*

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and *Vrn-D5*, which confer low sensitivity to vernalization (Pugsley, 1971; 1972). Non-fulfillment of vernalization requirement results in delayed flowering of sensitive wheat cultivars (Levy & Peterson, 1972; Jedel et al., 1986).

Knowledge of the nature of gene action controlling flowering and maturity times of spring wheat may aid in formulating breeding strategies to modify these traits according to the needs of a given growing environment. Several studies have demonstrated the involvement of additive, dominance or epistatic gene actions in the inheritance of heading time of field-grown wheat. Klaimi and Qualset (1974) reported the involvement of additive, dominance and epistatic gene action in controlling heading time of spring wheat. Nanda et al. (1981), Bhatt (1972) and Sheikh et al. (2000) concluded that additive gene action was more important than dominant gene action in the inheritance of heading time in spring wheat. The importance of both additive and dominance effects in controlling heading time has been reported in winter (Edwards et al., 1976) and spring (Singh et al., 2003) wheat.

The diallel cross has been widely used to provide information on the general combining ability (GCA) of parents and the specific combining ability (SCA) of crosses; as well as other genetic parameters such as variance components and heritability estimates (Xiang & Li, 2001). Most of the genetic analysis models of diallel cross data assume epistatic genetic effects to be absent, which in fact may not be true for complex traits such as heading and maturity time in wheat. For detecting additive, dominance and additive × additive epistatic effects, Zhu (1994) developed an additive-dominance-epistasis (ADAA) genetic model that requires data from parental, F_1 and F_2 generations. The use of such models may help improve the wheat breeder's understanding of the underlying gene action, and provide a better estimate of the heritability of a given trait.

Presently, there is limited knowledge of the specific genes governing the range of maturity in western Canadian adapted spring wheat cultivars. Cold temperatures at planting and in early growth stages may alter vernalization pathways in responsive spring wheat genotypes. Due to inconsistent vernalization conditions in western Canada,

vernalization responsive spring wheat genotypes may exhibit variable days to maturity and yield potential (Jedel, 1994). Recent advances in wheat genomics have resulted in the cloning of three major vernalization genes, *Vrn-A1*, *Vrn-B1* and *Vrn-D1* (Yan et al., 2003). Polymerase chain reaction (PCR) markers are now available that can facilitate the rapid characterization of wheat germplasm for *Vrn* genes and hence lead to a better understanding of the adaptive value of different *Vrn* alleles in a particular growing region (Yan et al., 2004; Fu et al., 2005).

A better understanding of the underlying genetic control of flowering/maturity time in western Canadian adapted spring wheats may aid in breeding for early maturity. The present study was conducted to, 1) investigate the relative importance of additive, dominance and epistatic genetic effects on the inheritance of time to flowering and maturity in a one-way diallel cross among western Canadian adapted spring wheat cultivars under field conditions, and 2) determine the presence and number of different vernalization response genes from segregation analyses of the F_2 generations as well as from molecular based approaches.

3.2 Materials and Methods

Five spring wheat cultivars spanning the range in maturity of western Canadian adapted spring common wheat were randomly selected for this study. The cultivars included 'AC Taber' (late), 'AC Foremost' (late), 'AC Barrie' (medium), 'AC Intrepid' (early) and 'Cutler' (early). The five cultivars were crossed in a one-way diallel mating design to obtain a total of 10 cross combinations. Ten F_1 seeds from each of the 10 crosses were grown in the greenhouse and were allowed to self-pollinate. The F_2 seeds were harvested in bulk.

The F_1 crosses and F_2 populations were field-evaluated along with the 5 parents at the Edmonton Research Station of the University of Alberta, Edmonton, (53°34′N, 113°31′W) during the summers of 2004 and 2005. The experiment was planted relatively late, May 17 in 2004 and May 26 in 2005, in an attempt to avoid frost damage and vernalization of the plants. Soils at the experimental site were Orthic Black Chernozems

(AAFRD, 2004). The experiment, in each year, was laid out in a split-plot arrangement of four randomized complete blocks. The F_1 and F_2 generations were assigned to the main plot with crosses and parents within generations assigned to subplots. The F_1 diallel was hand seeded in a single row plot, 3 m long with plant spacing of 15 cm and row spacing of 30 cm (21 seeds row^{-1}). The F_2 diallel was hand seeded in 5-row plots with plant and row spacing similar to that of F_1s (105 seeds exp. unit^{-1}). Hence, each block consisted of 90 rows, 15 for the F_1 (1 row each for 5 parents and 10 crosses) and 75 for the F_2 (5 rows each for 5 parents and 10 populations) diallel. The experiment was irrigated with overhead sprinklers, when needed.

Ten plants were randomly chosen in each row of F_1 crosses and parents, and observations were made on the number of days from seeding to anthesis and maturity, plant height and grain yield plant^{-1}. Twenty five plants (5 each row) were randomly selected in the F_2 diallel for making observations on the same traits, for quantitative genetic analyses. For segregation analyses of the F_2 populations, all F_2 plants were scored for days to anthesis. Anthesis date was recorded when 50% of the heads on a plant started dehiscing anthers. Physiological maturity was visually determined when 50% of the peduncles on a plant had completely lost green colour. The number of calendar days from seeding to anthesis and maturity, in each year, was converted to growing degree days by summing the average daily temperatures (over a base temperature of 0°C) from the date of seeding to the date when anthesis or maturity was recorded. Grain fill duration was calculated as the difference between days to maturity and anthesis.

Combined analyses of variance on the F_1 and F_2 data were performed in the MIXED procedure of SAS (SAS Institute, 2003) to test if F_1 and F_2 means were equal. All effects (Year, Block (Year), Generation × Year, Genotype, Generation × Genotype, Genotype × Year, Generation × Genotype × Year) except Generation were considered random. Heterogeneity of error variance was accounted for by including the REPEATED/GROUP=YEAR statement (Piepho, 1999). Likelihood ratio testing was used to test if individual variance components were zero. Likelihood ratios were constructed as differences between the -2 Residual Log Likelihood values of the reduced

covariance model (without the effect being tested) and the full covariance model (with the effect being tested) (Yang, 2002). Likelihood ratio test probabilities were halved prior to comparing with Chi-square tabulated value (Self & Liang, 1987). Due to significant Generation × Genotype × Year effects for all traits except plant height, data were analyzed BY GENERATION, which revealed significant Genotype × Year effects for all traits except plant height in the F_2. Further analyses of the F_1 and F_2 diallels were, therefore, performed separately for each year. The genotype effects were further partitioned into parents and crosses (using BY GENOTYPE statement), and parents versus crosses (using the ESTIMATE statement). For the purpose of estimating genotypic performances, best linear unbiased predictors (BLUPs) were obtained.

Diallel analyses were performed on the mean values of parents, F_1 crosses and F_2 populations, employing an Additive-Dominance-AdditivexAdditive (ADAA) genetic model for the combined analyses of F_1 and F_2 diallels (Zhu, 2003). The genetic variance components were estimated based on an ADAA model using a mixed linear model approach, minimum norm quadratic unbiased estimation (MINQUE) (Rao, 1971). The genetic effects were predicted using the Adjusted Unbiased Prediction (AUP) method (Zhu & Weir, 1996). Jackknifing over genotype (4 reps for each genotype) was used to estimate standard errors of variances and the predicted genetic effects (J. Zhu, personal communication). Narrow-sense heritability across environments was estimated as $h_N^2 = (V_A + V_{AA})/V_P$, and broad-sense heritability across environments as $h_B^2 = (V_A + V_{AA} + V_D)/V_P$. The significances of variance components were tested using one-tailed *t*-tests, whereas those of genetic effects were tested using two-tailed *t*-tests. All genetic analyses were performed in the software 'QGAStation 1.0' (Chen & Zhu, 2003). For segregation analyses of the F_2 populations, plants were classified into spring (those reaching anthesis) and winter types (those remaining vegetative 100 days after planting). The number of plants in each class was used for fitting to the expected ratios based on the number of genes segregating.

For determining the *Vrn* genotype of the parental genotypes, genomic DNA was extracted from 7-10 day old plants of the five cultivars and three check genotypes TD-B,

TD-D and TD-E, known to carry dominant alleles at the *Vrn-B1*, *Vrn-A1* and *Vrn-D1* loci, respectively (Pugsley, 1971). Extract-N-Amp™ Plant PCR Kit (Sigma-Aldrich, Cat# XNAP) was used for genomic DNA extraction following the protocol provided by the manufacturer. PCR primers reported in Yan et al. (2004) and Fu et al. (2005) were used to detect the presence of dominant or recessive alleles of *Vrn-A1*, *Vrn-B1* and *Vrn-D1*. PCR was performed in a 20μL volume in a GeneAmp® 9700 thermocycler (Applied Biosystems). The reaction mixture contained 0.5 μL each of the 5 μM forward and reverse primers, 10 μL Extract-N-Amp™ PCR ReadyMix (Sigma-Aldrich, Cat# E3004), 5 μL sterile water and 4 μL DNA extract. Cycling parameters and annealing temperatures were the same as Yan et al. (2004) and Fu et al. (2005). PCR products were visualized on 1.5% agarose gel stained with ethidium bromide. The *Vrn* genotypes of the parents were confirmed from two independent PCR.

3.3 Results

The difference between F_1 and F_2 generations was not significant for any trait in the combined (over years and generations) analyses but was significant for yield plant^{-1} in 2005 (results not shown). Combined analyses (over years) revealed significant ($P<0.05$) genotype × year interaction for all traits except plant height in the F_2 diallel (results not shown). Results are, therefore, presented with reference to individual years hereafter. Genotypes differed ($P<0.01$) for all traits measured on F_1 and F_2 generations in both years (Table 3.1). Genotypes accounted for more than 50% of the total variation in all traits except grain fill duration (F_2) in 2004 and yield plant^{-1} (F_1) in 2005 (Table 3.1). Among the components of genotypic variance in the F_1 diallel, parents and crosses were significant ($P<0.05$) for all traits except yield plant^{-1} in 2005, whereas parents vs. crosses effects were significant ($P<0.05$) for all traits except days to anthesis in 2005. The effect of parents in the F_2 diallel was significant for all traits except grain fill duration in 2004 and yield plant^{-1} in both years. The effect of F_2 crosses was significant for all traits except plant height in 2005. Parents vs. crosses effect in the F_2 diallel was significant for all traits except days to anthesis and yield plant^{-1} in 2005. 'Taber' was the latest of the five cultivars followed by 'Foremost', 'Barrie', 'Cutler' and 'Intrepid' in decreasing order of days to anthesis/maturity (Table 3.2). The F_1 and F_2 crosses involving 'Taber'

flowered/matured later than those that did not have 'Taber' as one of the parents (Table 3.2). All the genotypes flowered (and most also matured) comparatively earlier in 2005 than in 2004. Similarly, all genotypes yielded higher in 2005 than in 2004. The lower yields in 2004 were probably due to freezing temperatures and snowfall in September.

Significant (*P<0.01*) additive genetic effects were observed for days to anthesis and maturity in both years, for plant height in 2005 and for yield plant^{-1} in 2004 (Table 3.3). Additive genetic effects accounted for >50% of the total phenotypic variation in days to anthesis and maturity in both years, but were higher in magnitudes in 2005 than in 2004 (Table 3.3). Significant (*P<0.05*) dominance effects were also detected for days to anthesis and maturity in both years, with greater effect in 2004. Additive × additive (A×A) epistatic effect was significant (*P<0.05*) but small in magnitude for days to maturity in 2005. Significant (*P<0.05*) dominance and A×A epistatic effects were detected for grain fill duration, plant height and yield plant^{-1} in one or both years. Dominance effects were more important than epistatic effects in controlling grain fill duration in both years, indicating that early generation selection will not be effective in modifying this trait. The magnitudes of dominance and epistatic effects for plant height varied over years (Table 3.3). Dominance effects accounted for >50% of the total phenotypic variation in yield plant^{-1} in both years, suggesting that selection should be delayed until later generations to obtain genetic gain in this trait. Significant (*P<0.05*) A×A epistasis was observed for yield plant^{-1} in 2005. Narrow-sense heritabilities were medium to high (60-86%) for days to anthesis and maturity, but low to medium (13-55%) for grain fill duration, plant height and yield plant^{-1} (Table 3.3). With the exception of plant height, narrow-sense heritabilities were higher for all traits in 2005 than in 2004. Broad-sense heritabilities were high (>75%) for all traits.

Significant (*P<0.05*) general combining abilities (GCAs) for days to anthesis and maturity were observed for all parents (Table 3.4). 'Taber' and 'Foremost' exhibited positive, while 'Barrie', 'Cutler' and 'Intrepid' exhibited negative GCA effects. 'Taber' exhibited the highest positive GCA for days to anthesis in 2004 and 2005 (53 and 66) and also had the highest positive GCA for days to maturity (55) in both years (Table 3.4). The

two early maturing cultivars ('Cutler' and 'Intrepid') had the highest negative GCAs for days to anthesis, with the latter also having the highest negative GCA for days to maturity. General combining abilities were not significant ($P \geq 0.05$) for grain fill duration and plant height in both years, and yield plant^{-1} in 2005. Significant ($P < 0.05$) positive specific combining ability (SCA) effects for days to anthesis were detected for the cross 'Taber × Foremost' (46) in both years and 'Barrie × Intrepid' (23) in 2005, whereas there was significant negative SCA (-56) for 'Taber × Cutler' in 2005 (Table 3.4). Significant ($P < 0.05$) positive SCAs were observed for days to maturity for 'Taber × Barrie' (16) and 'Cutler × Foremost' (8) in 2005 and 'Taber × Foremost' (57) in 2004. Significant negative SCA effects for days to maturity were observed for 'Taber × Cutler' (-21) and 'Foremost × Intrepid' (-40) in 2004 and for 'Barrie × Intrepid' (-16) in 2005. Significance of SCA indicates the involvement of non-additive genetic effects in these crosses.

Frequency distributions of days to anthesis in the F_2 populations of all crosses were skewed (Figures 3.1, 3.2). The means of the F_2 populations of all crosses were in or out of the range of the late parents and generally shifted towards lateness. Based on the segregation behaviour in the F_2, the five parents fell into two distinct groups, with 'Taber' and 'Foremost' being one and 'Barrie', 'Cutler' and 'Intrepid' being the other group. Crosses between groups produced winter type segregants in the F_2 generation with no exceptions. Crosses within groups did produce some transgressively late segregants but no winter types. The occurrence of winter types in the F_2 of crosses between groups indicated that the vernalization responses of parents in each cross were governed by different loci. The segregation patterns in the crosses between groups fitted the expected ratio of 15:1 for two gene models for all crosses in both years (Table 3.5). The F_2 frequency distributions of crosses between groups were discontinuous suggesting the segregation of few major genes. The crosses 'Taber × Foremost', 'Cutler × Barrie', 'Cutler × Intrepid' and 'Barrie × Intrepid' failed to segregate into winter types, indicating that vernalization response of parents in each cross was governed by a common *Vrn* gene. The frequency distributions of these crosses were continuous (Figures 3.1, 3.2) indicating the segregation of modifier (earliness *per se*) genes.

Amplification of genomic DNA using VRN1AF and VRN1R primers showed the presence of two PCR products of approximately 650 and 750 bp in 'Cutler', 'Barrie', 'Intrepid' and TD-D, and a PCR product of 500 bp in 'Taber', 'Foremost', TD-B and TD-E (Figure 3.3). This indicates that 'Cutler', 'Barrie' and 'Intrepid' carry the dominant allele *Vrn-A1* while 'Taber' and 'Foremost' carry the recessive allele *vrn-A1*. Primers Intr1/B/F and Intr1/B/R3 produced an amplification product in TD-B, 'Taber' and 'Foremost' but not in 'Cutler', 'Barrie' and 'Intrepid'. Hence, 'Taber' and 'Foremost' carry the dominant allele *Vrn-B1* while 'Cutler', 'Barrie' and 'Intrepid' carry the recessive allele *vrn-B1*. Primers Intr1/D/F and Intr1/D/R3 amplified a PCR product (band close to 1650 bp) only in TD-E suggesting that none of the five cultivars carry the dominant allele *Vrn-D1*.

3.4 Discussion and Conclusions

Flowering time in wheat is genetically controlled by vernalization and photoperiod response, and earliness *per se,* genes. Photoperiod response is believed to provide a more stable control of flowering time as it does not vary with year (Levy & Peterson, 1972). Wheat breeders at high northern latitudes, however, cannot exploit photoperiodic heading response to a larger extent because the photoperiod requirement in these areas is most likely fulfilled during the growing season (Marshall et al., 1989; Worland et al., 1998). Segregation analyses of the F_2 generations in the present study indicated the presence of two different vernalization (*Vrn*) genes in western Canadian adapted spring wheats. This was further confirmed through molecular genetic analyses which revealed that the spring growth habit of 'Cutler', 'Barrie' and 'Intrepid' is determined by dominant allele of *Vrn-A1* and that of 'Taber' and 'Foremost' by the dominant *Vrn-B1*. The overall genetic effect on time to anthesis/maturity was mainly additive. The preponderance of additive genetic effects suggests that selection for early flowering/maturity in early generations will result in genetic improvement towards earliness.

The appearance of winter type segregates in the F_2 generations of some crosses and molecular genetic analysis, revealed the presence of vernalization non-responsive

Vrn-A1 as well as vernalization responsive *Vrn-B1* in western Canadian adapted spring wheats. Vernalization responsive genes are known to contribute indirectly to yield by influencing flowering time (Flood & Halloran, 1986), tiller (Levy & Peterson, 1972) and spikelet number (Gororo et al., 2001; Whitechurch & Snape, 2003) in sensitive genotypes. The yield advantage of vernalization responsive cultivars appears to be the reason for the adoption of such cultivars in high northern growing regions. The incorporation of *Vrn-D1* into spring wheat was strongly recommended as it confers wide adaptation and high yield potential (Stelmakh, 1993).

A recent study concluded that *Vrn-D1* had the highest frequency among the major *Vrn* genes in the globally important CIMMYT wheat cultivars, with the semi-dwarf Mexican cultivar 'Sonora 64' as one of the donors responsible for the wide distribution of *Vrn-D1* gene (van Beem et al. 2005). However, characterization of the spring wheat cultivars used in the present study and some other high northern latitude adapted spring wheat cultivars (unpublished) for *Vrn* genes indicates that the predominant vernalization responsive gene in these regions is *Vrn-B1*, and that the frequency of vernalization non-responsive *Vrn-A1* is very high. These results agree with Stelmakh (1998), who reported that *Vrn-A1* is present in the highest frequencies in high latitudes, followed by *Vrn-B1*. The crosses of 'Barrie' with 'Cutler' and 'Intrepid' did not produce winter type segregants in F_2 due to the common presence of dominant *Vrn-A1* allele. Nevertheless, some segregants from these crosses were up to 10 days later than the late parent. This variation in flowering time appears to be due to the segregation of earliness *per se* genes that affect flowering/maturity time independently of *Vrn* and *Ppd* genes. Previous results demonstrated that 'Barrie' flowers significantly later than the rest of the cultivars in this study when vernalized and grown in a long photoperiod (Chapter 2).

Additive genetic effects are important in formulating breeding strategies. In the present study, additive genetic effects played a major role in the inheritance of time to flowering/maturity in western Canadian adapted spring wheats. Dominance also affected time to flowering/maturity, but was of little importance due to its smaller effects and significant interaction with year. The additive effects, on the contrary, did not interact

with years and had greater effect on the two traits. The major role of additive genetic effects in controlling flowering/maturity suggests that selection in early segregating generations should be effective in bringing about desirable changes in these traits. The involvement of additive effects in the inheritance of heading/maturity time in spring wheat was previously reported by Bhatt (1972), Klaimi & Qualset (1974), Nanda et al. (1981), Sheikh et al. (2000) and Singh et al. (2003). Epistatic effects were also important in controlling heading/maturity time of spring wheat (Klaimi & Qualset, 1974; Nanda et al., 1981).

Vernalization non-responsiveness is believed to be a preferred phenotype for the short- growing season of western Canada (Jedel, 1994) to avoid inconsistent maturity and yield patterns. Wheat breeders in high northern latitudes have incorporated the vernalization insensitive gene *Vrn-A1* into spring wheats by selecting for early flowering/maturing genotypes that complete their life cycles within the short growing season, hence avoiding early and late season frosts. This earliness of flowering/maturity is achieved at the cost of relatively low grain yields. Within wheat growing sub-regions of high northern latitudes where frosts normally occur comparatively late in the fall, vernalization responsive wheat cultivars offer a yield advantage over the early maturing cultivars. This yield advantage, however, may not always be fully realized due to the highly variable climatic conditions experienced in higher northern latitudes. Within these two extremes, a combination of *Vrn* genes might offer a possibility for developing early maturing wheat cultivars with higher yield potential based on the known genetic effects of different *Vrn* genes on earliness and other agronomic traits (Stelmakh, 1993). Stelmakh (1993), working with isogenic lines of different combinations of dominant *Vrn* alleles, reported the triple dominant *Vrn* genotype to be the earliest but low yielding. Combinations of dominant *Vrn-A1* with either dominant *Vrn-B1* or dominant *Vrn-D1* were early and high yielding. Stelmakh (1993) further reported that dominant *Vrn-B1* or dominant *Vrn-D1* either singly or in combination, were high yielding but late maturing.

The present study revealed the presence of spring habit alleles at *Vrn-A1* and *Vrn-B1* loci in western Canadian adapted spring wheats. The spring habit allele at *Vrn-B1*

locus confers slight sensitivity to vernalization, and results in delayed flowering/maturity under non-vernalizing growing conditions. Results of this study indicate the importance of knowing the *Vrn* genes of wheat germplasm that may be used as parents in breeding programs for earliness in western Canada. Due to the availability of PCR based markers for the major vernalization genes, large numbers of germplasm can be readily characterized for the presence of desirable *Vrn* genes/combinations which may be tracked throughout the segregating generations in a breeding program. Besides vernalization response, photoperiod sensitivity/insensitivity, which may not greatly affect flowering/maturity times unless accompanied with lateness *per se*, may also be exploited in breeding high yielding spring wheat cultivars for higher northern latitudes (Dyck et al., 2004; Knott, 1986). Once desirable vernalization and photoperiod responses are obtained, earliness *per se* genes may be incorporated to further modify flowering/maturity times of spring wheat in higher northern growing regions. The use of PCR based markers for *Vrn* and *Ppd* genes, and closely linked markers to earliness *per se* genes, will greatly increase the efficiency of germplasm characterization and selection for desirable flowering/maturity time.

3.5 Summary

Due to the short growing season in western Canada, the development of early maturing spring wheat (*Triticum aestivum* L.) cultivars is important to avoid frost damage which can lower production and quality. Earliness of flowering and maturity, and some associated agronomic traits, were investigated using a set of randomly selected western Canadian adapted spring wheat cultivars (differing in maturity) and their F_1 and F_2 progeny made in a one-way diallel mating design. The parents, and their F_1 and F_2 progeny were evaluated under field conditions over two years. Anthesis and maturity times were controlled by both vernalization response and earliness *per se* genes, mainly acting additively. Non-additive genetic effects were more important in controlling grain fill duration, grain yield and plant height. Additive × additive epistatic effects were detected for all traits studied except time to anthesis. Segregation analyses of the F_2 populations for time to anthesis indicated the presence of different vernalization response genes. Molecular genetic analyses revealed the presence of *Vrn-A1* and *Vrn-B1* genes in

the parental cultivars. Narrow-sense heritability was medium to high (60-86%) for anthesis and maturity times but low to medium (13-55%) for grain fill duration, plant height and grain yield. Selection for early flowering/maturity in early segregating generations would be expected to result in genetic improvement towards earliness in western Canadian spring wheats. Wheat germplasm should be characterized for *Vrn* genes before using them as parents in breeding programs for earliness in western Canada.

3.6 Tables and Figures

Table 3.1. Analyses of variance for five agronomic traits of a one-way diallel cross among five spring wheat cultivars differing in time to maturity.

Source	Variance Components (% of total)									
	Anthesis		Maturity		GFD[y]		Plant Height		Yield Plant^{-1}	
	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
a) F_1										
Block	15**	1^{ns}	19**	3**	12**	5*	6**	24**	19**	15**
Genotype	73**	94**	69**	89**	71**	78**	88**	61**	51**	30*
Parents (P)[x]	73**	68**	67**	74**	83**	58**	73**	73**	66**	71^{ns}
Crosses (C)[x]	27**	32**	33**	26**	17**	42**	27**	27**	38**	29^{ns}
P vs. C	*	*ns*	**	**	**	**	**	**	*	**
Residual	12	5	12	8	17	18	6	15	30	55
b) F_2										
Block	9**	0	20**	0	27**	3^{ns}	0	5^{ns}	0	0
Genotype	75**	93**	65**	88**	40**	70**	90**	58**	61**	66**
Parents (P)[x]	55**	72**	59**	76**	42^{ns}	63**	80**	78**	39^{ns}	78^{ns}
Crosses (C)[x]	45**	28**	41**	28**	58**	37**	20**	22^{ns}	61**	22**
P vs. C	**	*ns*	**	**	*	**	**	**	**	*ns*
Residual	16	7	15	12	33	27	10	37	39	34

**,* Significant at $P<0.01$, and $P<0.05$, respectively based on likelihood ratio test.
[ns] Not significant ($P\geq0.05$).
[y]Grain Fill Duration.
[x]Proportions of the sum of parents and crosses.

Table 3.2. Best Linear Unbiased Predictors (BLUPs) of genotypic performance for three agronomic traits in a one-way diallel cross among five spring wheats differing in maturity.

Genotype	F_1						F_2					
	Anthesis (°C days)		Maturity (°C days)		Yield Plant^{-1} (gm)		Anthesis (°C days)		Maturity (°C days)		Yield Plant^{-1} (gm)	
	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
Parents												
Taber	1113	1071	1843	1795	30.0	33.3	1086	1066	1777	1779	29.2	31.9
Cutler	932	847	1639	1608	26.6	31.9	920	845	1629	1621	31.5	31.7
Foremost	1053	969	1763	1747	27.5	39.5	1019	974	1730	1742	26.9	41.8
Barrie	1017	923	1701	1693	27.0	31.9	974	910	1657	1668	27.6	32.6
Intrepid	926	853	1633	1564	24.8	29.6	915	855	1619	1560	25.7	29.2
Crosses												
Tab × Cut	1018	980	1750	1748	28.6	32.5	1065	928	1760	1724	28.9	33.2
Tab × For	1143	1078	1881	1789	26.9	30.3	1112	1043	1826	1761	29.7	32.3
Tab × Bar	1090	985	1815	1745	26.8	32.7	1038	1018	1748	1773	29.6	33.1
Tab × Int	1043	951	1776	1711	26.2	32.4	1033	951	1737	1699	26.9	38.8
Cut × For	986	906	1706	1721	29.0	28.8	1030	917	1736	1711	25.0	32.8
Cut × Bar	971	883	1693	1664	26.8	28.3	953	888	1661	1654	26.9	30.7
Cut × Int	1000	858	1710	1610	24.5	29.4	972	863	1692	1645	27.9	31.8
For × Bar	1020	942	1741	1694	25.7	30.1	1020	942	1736	1690	25.4	29.3
For × Int	991	916	1687	1675	24.7	29.5	1008	950	1715	1699	25.5	32.1
Bar × Int	993	894	1678	1629	24.5	28.6	987	874	1665	1605	23.8	30.2
$SE_{difference}$	19	12	24	15	1.0	1.3	22	13	22	17	1.0	1.7

Table 3.3. Proportions (%) of variance components to phenotypic variance for five agronomic traits in a one-way diallel cross (F_1 and F_2) among five spring wheats differing in maturity.

Parameter[y]	Anthesis		Maturity		Grain Fill Duration		Plant Height		Yield Plant^{-1}	
	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
V_A/V_P	56**	77**	61**	70**	0	0	0	6**	10**	0
V_D/V_P	23**	15**	24**	7*	57**	43**	32**	51**	66**	57**
V_{AA}/V_P	4^{ns}	1^{ns}	0	11*	22**	33**	55**	23**	3^{ns}	31**
V_e/V_P	17	7	15	12	21	24	13	20	21	13
h_N^2	60**	78**	61**	81**	22**	33**	55**	29**	13*	31**
h_B^2	83**	93**	85**	86**	79**	76**	87**	80**	78**	87**

**, * Significantly different from zero at $P<0.01$ and $P<0.05$, respectively; ^{ns} Not significant ($P\geq0.05$).

[y]V= Variance, P=Phenotypic, A=Additive, D=Dominance, AA= Additive×Additive epistasis, e= Residual;

h_N^2 and h_B^2 are narrow and broad-sense heritabilities.

Table 3.4. General combining abilities (GCA) of parents and specific combining abilities of F_1 and F_2 crosses for three traits in a one-way diallel cross among five spring wheats.

Parent	Trait[y]	Cutler		Foremost		Barrie		Intrepid		GCA	
		2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
Taber	DAN	*ns*	-56*	43**	46**	*ns*	*ns*	*ns*	*ns*	53**	66**
	DMA	-21*	*ns*	57*	*ns*	*ns*	16*	*ns*	*ns*	55**	54**
	Yield	0.6*	0.8*	*ns*	-3*	*ns*	*ns*	*ns*	*ns*	1.1*	*ns*
Cutler	DAN			*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	-28*	-46**
	DMA			*ns*	8*	*ns*	*ns*	*ns*	*ns*	-28*	-19**
	Yield			6.1*	*ns*	*ns*	-3.5*	*ns*	*ns*	*ns*	*ns*
Foremost	DAN					*ns*	*ns*	*ns*	*ns*	23*	31**
	DMA					*ns*	*ns*	-40**	*ns*	30**	28**
	Yield					*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Barrie	DAN							*ns*	23*	-19**	-12*
	DMA							*ns*	-16*	-26**	-16*
	Yield							1.2*	*ns*	-0.4*	*ns*
Intrepid	DAN									-30**	-39**
	DMA									-39**	-47**
	Yield									-0.9**	*ns*

[y] Days to anthesis (DAN) and days to maturity (DMA).

**,* Significantly different from zero at $P<0.01$ and $P<0.05$, respectively.

[ns] Not significant ($P\geq0.05$).

Table 3.5. Segregation of F_2 plants for days to anthesis in ten crosses among five Canadian spring wheat cultivars differing in maturity.

Cross	2004				2005			
	No of F_2 plants		Segregation ratio (15:1)		No of F_2 plants		Segregation ratio (15:1)	
	Spring[y]	Winter[x]	χ^2	$P>\chi^2$	Spring[y]	Winter[x]	χ^2	$P>\chi^2$
Taber × Barrie	319	13	2.7	0.1	131	13	1.45	0.20
Taber × Cutler	262	26	3.34	0.05	193	20	3.07	0.05
Taber × Intrepid	264	17	0.97	0.25	155	14	0.87	0.25
Cutler × Foremost	281	21	0.15	0.5	213	13	0.03	0.75
Foremost × Barrie	297	11	3.33	0.05	208	17	0.45	0.50
Foremost × Intrepid	290	16	0.38	0.50	152	8	0.24	0.50
Taber × Foremost	267	0	-	-	184	0	-	-
Cutler × Barrie	350	0	-	-	246	0	-	-
Cutler × Intrepid	241	0	-	-	260	0	-	-
Barrie × Intrepid	295	0	-	-	299	0	-	-

[y] Plants reaching anthesis within 100 days after seeding.

[x] Plants remaining vegetative 100 days after seeding.

χ^2 value for significance at 5% level of probability is 3.84.

Figure 3.1. Frequency distributions for days to anthesis in the F_2 generation of ten crosses among five spring wheat along with the parental (represented by first letter), F_1 and F_2 means in 2004.

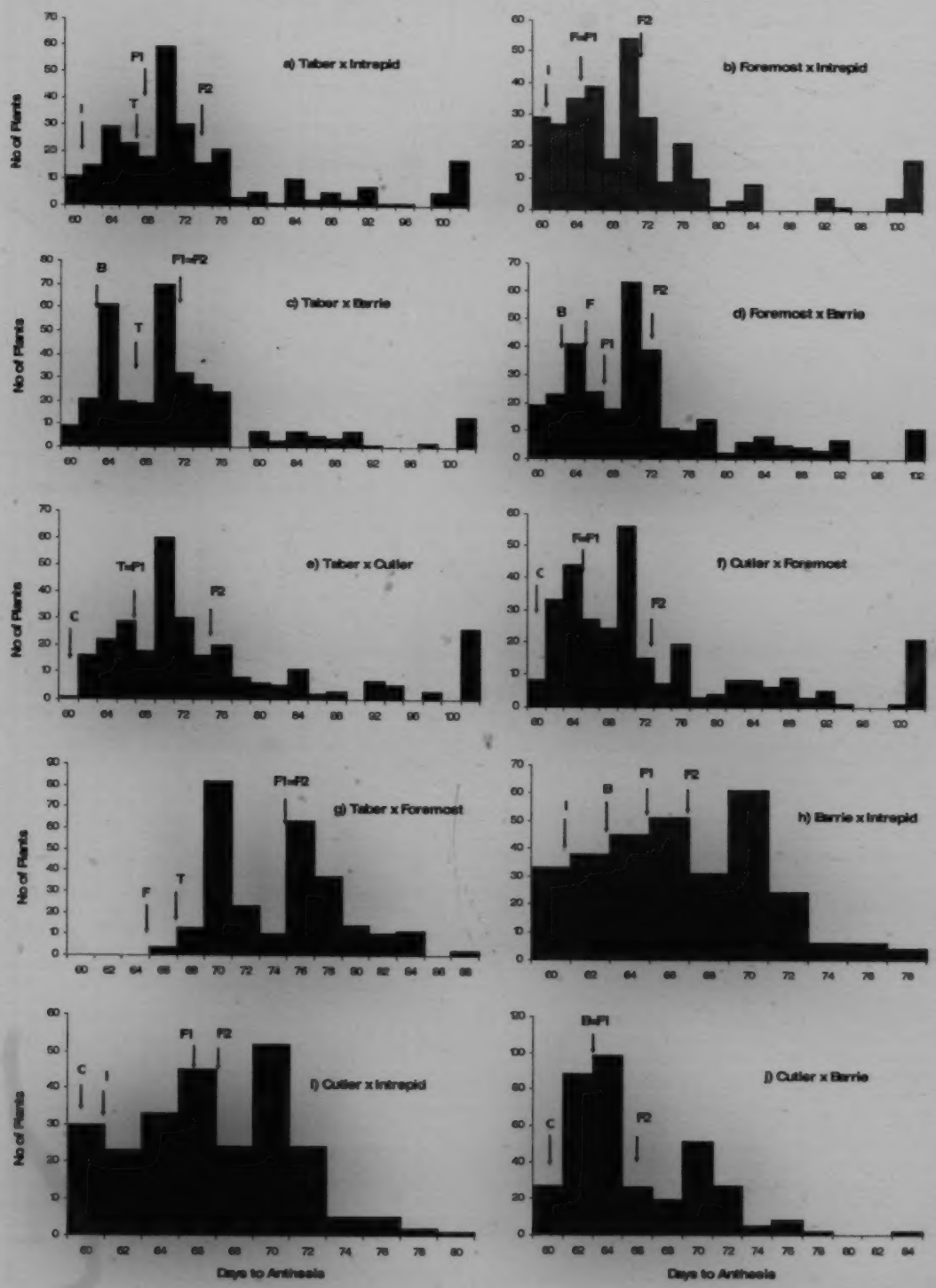
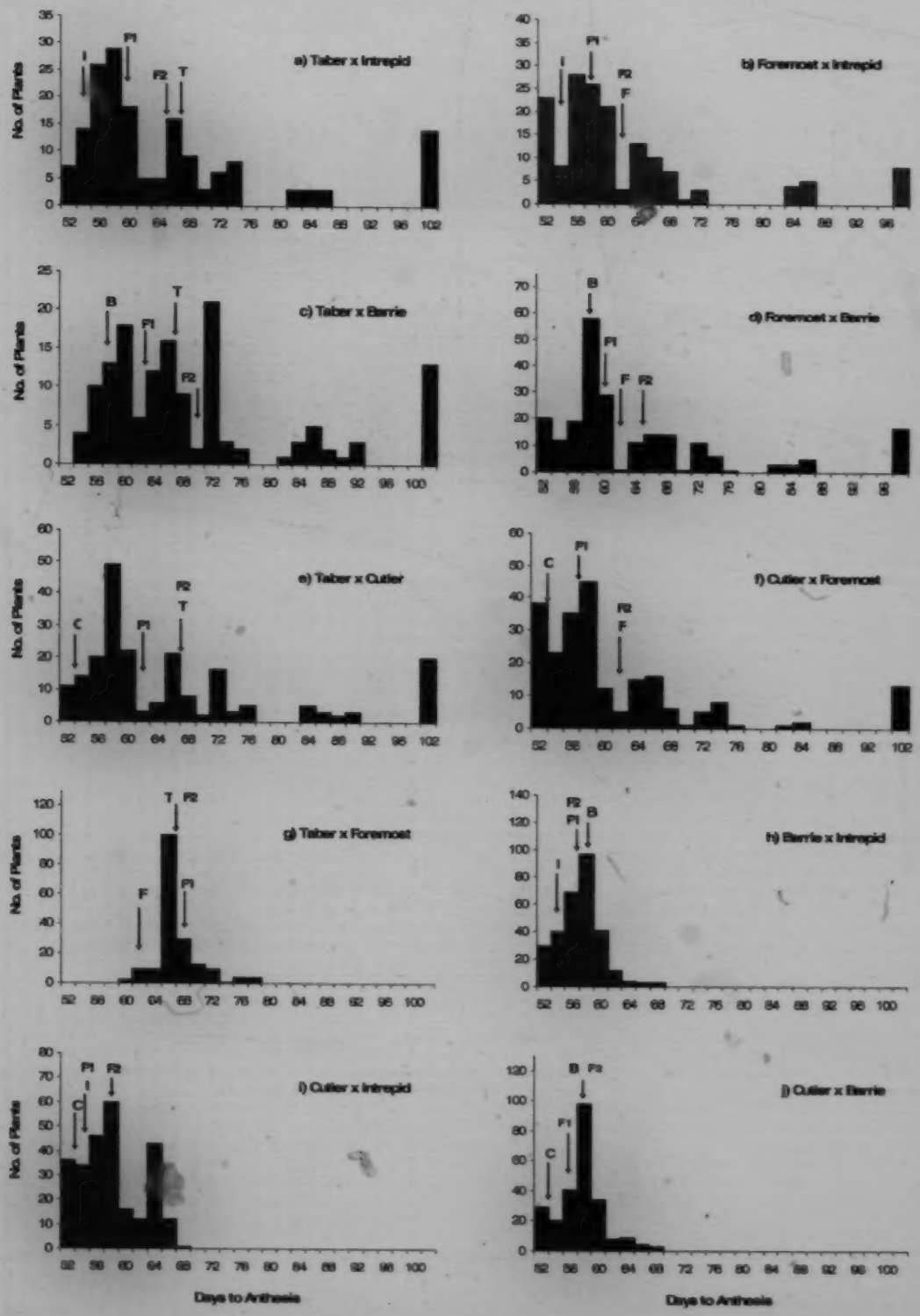


Figure 3.2. Frequency distributions for days to anthesis in the F_2 generation of ten crosses among five spring wheat along with the parental (represented by first letter), F_1 and F_2 means in 2005.



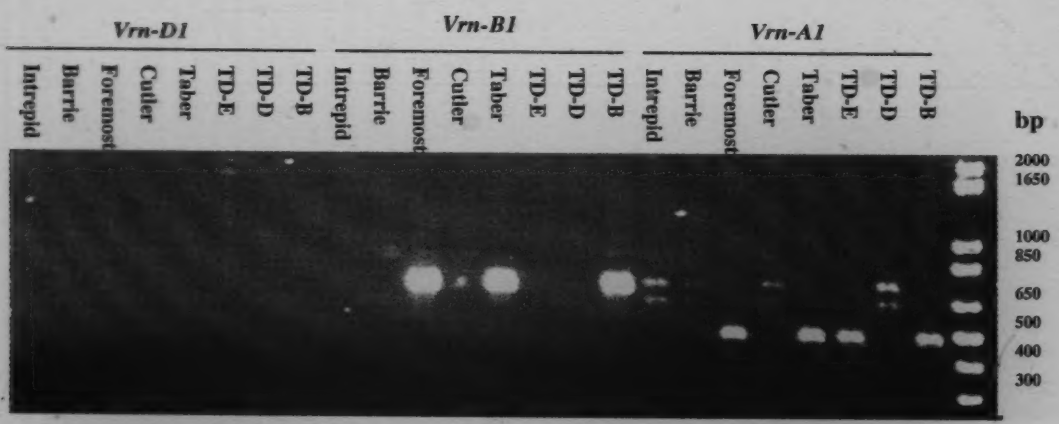


Figure 3.3. PCR amplification using genome specific primers VRN1AF/VRN1R (*Vrn-A1*), Intr1/B/F//Intr1/B/R3 (*Vrn-B1*) and Intr1/D/F//Intr1/D/R3 (*Vrn-D1*), for determining the *Vrn* genes of Canadian spring wheat. Known carriers of *Vrn-A1* (TD-D), *Vrn-B1* (TD-B) and *Vrn-D1* (TD-E) were used as controls.

3.7 References

Alberta Agriculture, Food and Rural Development (AAFRD), 2004. Soil Group Map of Alberta. [Online] http://www1.agric.gov.ab.ca/soils/soils.nsf/soilgroupmap?readform. [01 Feb 2006].

Bhatt, G.M. 1972. Inheritance of heading date, plant height, and kernel weight in two spring wheat crosses. Crop Sci. 12: 95-98.

Chen, G., and J. Zhu. 2003. 'QGAStation 1.0'. Software for the Classical Quantitative Genetics. Institute of Bioinformatics, Zhejiang University, China.

Dyck, J.A., M.A. Matus-Cadiz, P. Hucl, L. Talbert, T. Hunt, J.P. Dubuc, H. Nass, G. Clayton, J. Dobb, and J. Quick. 2004. Agronomic performance of hard red spring wheat isolines sensitive and insensitive to photoperiod. Crop Sci. 44: 1976-1981.

Edwards, L.H., H. Ketata, and E.L. Smith. 1976. Gene action of heading date, plant height, and other characters in two winter wheat crosses. Crop Sci. 16: 275-277.

Flood, R. G., and G.M. Halloran. 1986. Genetics and physiology of vernalization response in wheat. Adv. Agron. 39: 87-124.

Fu, D., P. Szűcs, L. Yan, M. Helguera, J.S. Skinner, J. von Zitzewitz, P.M. Hays, and J. Dubcovsky. 2005. Large deletions within the first intron in *VRN-1* are associated with spring growth habit in barley and wheat. Mol. Genet. Genomics, 273: 54-65.

Gororo, N.N., R.G. Flood, R.F. Eastwood, and H.A. Eagles. 2001. Photoperiod and vernalization responses in *T. turgidum* × *T. tauschii* synthetic hexaploid wheats. Ann. Bot. 88: 947-952.

Jedel, P.E. 1994. Inheritance of vernalization response in three populations of spring wheat. Can. J. Plant Sci. 74: 753-757.

Jedel, P.E., L.E. Evans, and R. Scarth. 1986. Vernalization responses of a selected group of spring wheat (*Triticum aestivum* L.) cultivars. Can. J. Plant Sci. 66: 1-9.

Kato, K., and H. Yamagata. 1988. Method for evaluation of chilling requirement and narrow-sense earliness of wheat cultivars. Japan. J. Breed. 38: 172-186.

Kato, K., and S. Yamashita. 1991. Varietal variation in photoperiodic response, chilling requirement and narrow-sense earliness and their relation to heading time in wheat (*Triticum aestivum* L.). Japan. J. Breed. 41: 475-484.

Klaimi, Y.Y., and C.O. Qualset. 1974. Genetics of heading time in wheat (*Triticum aestivum* L.). II. The inheritance of vernalization response. Genetics, 76: 119-134.

Knott, D.R. 1986. Effect of genes for photoperiodism, semi-dwarfism, and awns on agronomic characters in a wheat cross. Crop Sci. 26: 1158-1162.

Law, C. N., and A.J. Worland. 1997. Genetic analysis of some flowering time and adaptive traits in wheat. New Phytologist, 137: 19-28.

Levy, J., and M.L. Peterson. 1972. Responses of spring wheats to vernalization and photoperiod. Crop Sci. 12: 487-490.

Marshall, L., R. Busch, F. Cholick, I. Edwards, and R. Frohberg. 1989. Agronomic performance of spring wheat isolines differing for day length response. Crop Sci. 29: 752-757.

Nanda, G.S., G.N. Hazarika, and K.S. Gill. 1981. Inheritance of heading date, plant height, ear length and spikelets per spike in an intervarietal cross of wheat. Theor. Appl. Genet. 60: 167-171.

Ortiz-Ferrara, G., M.G. Mossad, V. Mahalakshmi, and R.A. Fischer. 1995. Photoperiod and vernalization response of wheat under controlled and field conditions. Plant Breed. 114: 505-509.

Piepho, H-P. 1999. Stability analysis using the SAS system. Agron. J. 91: 154-160.

Poehlman, J.M., and D.A. Sleper. 1995. Breeding Field Crops. Iowa State University Press, Ames, Iowa, USA, p-151.

Pugsley, A.T. 1971. A genetic analysis of the spring-winter habit of growth in wheat. Aust. J. Agric. Res. 22: 21-31.

Pugsley, A.T. 1972. Additional genes inhibiting winter habit in wheat. Euphytica 21: 547-552.

Rao, C.R. 1971. Estimation of variance and covariance components-MINQUE theory. J. Multivariate Analysis, 1: 257-275.

SAS Institute. 2003. Release 9.1. SAS Institute, Inc., Cary, NC, USA.

Self, S.G., and K-Y. Liang. 1987. Asymptotic properties of maximum likelihood estimators and likelihood ratio tests under nonstandard conditions. J. Am. Stat. Assoc. 82: 605-610.

Sheikh, S., I. Singh, and J. Singh. 2000. Inheritance of some quantitative traits in bread wheat (*Triticum aestivum* L. em Thell.). Ann. Agric. Res. 21: 51-54.

Singh, H., S.N. Sharma, R.S. Sain, and D.L. Singhania. 2003. The inheritance of production traits in bread wheat by diallel analysis. SABRAO J. 35: 1-9.

Stelmakh, A.F. 1993. Genetic effect of *Vrn* genes on heading date and agronomic traits in bread wheat. Euphytica, 65: 53-60.

Stelmakh, A.F. 1998. Genetic systems regulating flowering response in wheat. Euphytica, 100: 359-369.

van Beem, J., V. Mohler, R. Lukman, M. van Ginkel, M. William, J. Crossa, and A.J. Worland. 2005. Analysis of genetic factors influencing the developmental rate of globally important CIMMYT wheat cultivars. Crop Sci. 45: 2113-2119.

Whitechurch, E. M., and J.W. Snape. 2003. Developmental responses to vernalization in wheat deletion lines for chromosomes 5A and 5D. Plant Breed. 122: 35-39.

Worland, A.J., A. Borner, V. Korzun, W.M. Li, S. Petrovic, and E.J. Sayers. 1998. The influence of photoperiod genes on the adaptability of European winter wheats. Euphytica, 100: 385-394.

Xiang, B., and B. Li. 2001. A new mixed analytical method for genetic analysis of diallel data. Can. J. For. Res. 31: 2252-2259.

Yan, L., A. Loukoianov, G. Tranquilli, M. Helguera, T. Fahima, and J. Dubcovsky. 2003. Positional cloning of wheat vernalization gene *VRN1*. Proc. Natl. Acad. Sci. USA 100: 6263-6268.

Yan, L., M. Helguera, K. Kato, S. Fukuyama, J. Sherman, and J. Dubcovsky. 2004. Allelic variation at the *VRN-1* promoter region in polyploidy wheat. Theor. Appl. Genet. 109: 1677-1686.

Yang, R-C. 2002. Likelihood-based analysis of genotype-environment interactions. Crop Sci. 42: 1434-1440.

Zhu, J. 1994. General genetic models and new analysis methods for quantitative traits. J. Zhejiang Agric. Univ. 20: 551-559.

Zhu, J. 2003. Diallel analysis for an additive-dominance-epistasis model with genotype-by-environment interaction effects. In M.S. Kang (ed.): Handbook of Formulas and Software for Plant Geneticists and Breeders. Haworth press, New York, USA.

Zhu, J., and B.S. Weir. 1996. Mixed model approaches for diallel analysis on a bio-model. Genet. Res. (Camb.) 68: 233-240.

Chapter 4

Molecular Characterization of Vernalization Response Genes in Canadian Spring Wheat[4]

4.1 Introduction

Vernalization, or the "acquisition or acceleration of the ability to flower by a chilling treatment" (Chouard, 1960) is one of the important genetic factors affecting time to flowering/maturity in wheat (*Triticum aestivum* L.). Vernalization sensitivity/ insensitivity in wheat is controlled by alleles at the major vernalization loci, *Vrn-A1*, *Vrn-B1*, *Vrn-D1* and *Vrn-D5* (Pugsley, 1972). Winter wheat possesses recessive alleles at all loci, while spring wheat has dominant alleles at one or more of these loci. The dominant allele of *Vrn-A1* confers complete insensitivity to vernalization. It is epistatic to dominant alleles of *Vrn-B1*, *Vrn-D1* and *Vrn-D5*, which confer low sensitivity to vernalization (Pugsley, 1971; 1972). The differential vernalization requirements of the dominant alleles result in variation in the flowering time of spring wheat. This implies that, other genetic factors remaining the same, spring wheat having the dominant allele at *Vrn-A1* will be the first to flower, followed by those having dominant *Vrn-D1*, *Vrn-D5* and/or *Vrn-B1* (Goncharov, 2004). Stelmakh (1993, 1998) reported that *Vrn* genes have differential effects on heading time, plant height and yield components. Genotypes having two dominant alleles in combination at two vernalization loci tend to mature early and exhibit higher yield potential. Triple dominant genotypes were reported to be early but low yielding. This suggests the possibility of combining specific dominant *Vrn* alleles in spring wheat cultivars to improve grain yield potential while maintaining their earliness.

Recently, it has been demonstrated that mutations in the promoter region in the A genome of bread wheat are associated with dominant *Vrn-A1* alleles for spring growth habit (Yan et al., 2004), and that large deletions within the first intron at *Vrn-B1* and *Vrn-D1* loci are associated with dominant *Vrn-B1* and *Vrn-D1* alleles for spring growth habit (Fu et al., 2005). Polymerase chain reaction (PCR) based molecular markers have been developed to facilitate the rapid characterization of wheat germplasm for *Vrn* genes, and

[4] A version of this chapter has been submitted for publication to Genome.

to better understand the adaptive value of different *Vrn* alleles in different growing regions (Yan et al., 2004; Fu et al., 2005).

Due to the short growing season (95-125 days) in western Canada, the development of early maturing spring wheat cultivars is important to avoid frost damage which can lower production and quality (Tames, 2005). As the photoperiod requirement of wheat grown in these regions is most likely fulfilled due to long summer days (>14 hrs), wheat breeders may alter flowering time using vernalization response and earliness *per se* genetic mechanisms. Presently, there is limited knowledge of the specific genes determining genetic variation in flowering/maturity time of spring wheats grown in western Canada. The identification of vernalization response genes will allow for a greater understanding of the role of these genes in determining the flowering/maturity times of western Canadian adapted spring wheats. This may aid wheat breeders wishing to incorporate different *Vrn* genes into their germplasm, as one way of altering maturity without compromising yield potential.

The present study was conducted to identify the vernalization response genes (and hence to better understand the genetics of flowering/maturity time) of spring wheats grown in western Canada.

4.2 Materials and Methods

The plant material consisted of 42 western Canadian adapted spring wheat cultivars/lines (Table 4.1), representing spring wheat cultivars released in different eras, and 4 genotypes of known *Vrn* genes: Triple Dirk-B (*vrn-A1 Vrn-B1 vrn-D1 vrn-D5*), Triple Dirk D (*Vrn-A1 vrn-B1 vrn-D1 vrn-D5*), Triple Dirk E (*vrn-A1 vrn-B1 Vrn-D1 vrn-D5*) and Triple Dirk C (*vrn-A1 vrn-B1 vrn-D1 vrn-D5*) (Pugsley, 1971; 1972).

Three to four plants of each genotype were grown in a controlled environment chamber. Genomic DNA was extracted from leaves of 7-10 day old plants using the Extract-N-Amp™ Plant PCR Kit (Sigma-Aldrich, Oakville, Canada; Cat# XNAP), following the protocol provided by the manufacturer. Polymerase chain reaction primers

reported in Yan et al., (2004) and Fu et al., (2005) were used to detect the presence of dominant or recessive alleles of *Vrn-A1*, *Vrn-B1* and *Vrn-D1* genes (Table 4.2). Polymerase chain reaction was performed in a 20μL volume in a GeneAmp® 9700 thermocycler (Applied Biosystems; Foster City, CA). The reaction mixture contained 0.5 μL each of the 5 μM forward and reverse primers, 10 μL Extract-N-Amp™ PCR ReadyMix (Sigma-Aldrich, Cat# E3004), 5 μL sterile water and 4 μL DNA extract. After initial denaturation at 94°C for 4 min, 35-40 cycles at 94°C for 30 s, 55-61°C (depending on the primer pair used) for 30 s and 72°C for 2 min. were performed, followed by a final extension at 72°C for 10 min. PCR products were visualized on 1.5% agarose gel stained with ethidium bromide. The *Vrn* genotype of the cultivars/lines was confirmed from three independent PCR reactions.

The *Vrn* genes of four of the Canadian spring wheat cultivars (Taber, Foremost, Barrie and Intrepid) were also determined using segregation analyses in the F_2 generations. The four cultivars were crossed with each of the three isogenic lines of *Vrn* genes in the Triple Dirk background (TD-B, TD-D and TD-E, carrying spring habit alleles at *Vrn-B1*, *Vrn-A1*, and *Vrn-D1*, respectively). Ten F_1 seeds from each of the 12 crosses were grown in the greenhouse and were allowed to self-pollinate. The F_2 seeds were harvested in bulk. The F_2 plants were grown in 12.5 cm diameter pots (2 plants per pot) containing Sunshine-Mix (Sun Gro Horticulture Canada Ltd.) in a greenhouse maintained at 25°C on 6th June, 2006. The natural photoperiod (≈16 hrs) was extended by 2 hrs, through artificial illumination. Plants were watered when needed, and fertilized with water soluble commercial fertilizer (15-30-15: N-P_2O_5-K_2O) biweekly. Plants reaching heading within 75 days after planting were classified as spring, and those remaining vegetative at this stage as winter types. The number of plants in each class was used for fitting to the expected ratios based on the number of genes segregating.

4.3 Results

Amplification of genomic DNA using VRN1AF and VRN1R primers (*Vrn-A1*) exhibited the presence of two PCR products having similar sizes to that of TD-D in 35 of the 42 cultivars/lines (Figure 4.1). This indicates that these cultivars/lines carry the

dominant allele at the *Vrn-A1* locus. Six cultivars/lines produced a PCR product of similar size to TD-C, indicating the presence of the recessive allele *vrn-A1*. Primers Intr1/B/F and Intr1/B/R3 (*Vrn-B1*) produced an amplification product similar in size to that of TD-B in 21 of the cultivars/lines (Figure 4.1), indicating that these genotypes carry the dominant allele at the *Vrn-B1* locus. The absence of a PCR product in the remaining genotypes suggests that they carry the recessive allele at *Vrn-B1*. Primers Intr1/D/F and Intr1/D/R3 (*Vrn-D1*) amplified a PCR product (band close to 1650 bp) only in TD-E, suggesting the presence of a dominant allele at *Vrn-D1* locus. The absence of a PCR product in the remaining genotypes indicates that all 42 cultivars/lines tested carry the recessive allele of *Vrn-D1*. This also suggests that 'Rescue', CR5D, RC5A and RC5B (known to carry a dominant *Vrn* allele on chromosome 5D) (Major & Wheelan, 1985), carry *Vrn-D5* (formerly known as *Vrn4*) and not *Vrn-D1*.

The occurrence of winter type segregants in the F_2 generations of crosses between 'Taber' and TD-D, and 'Taber' and TD-E' suggested that 'Taber' carries winter habit alleles of *Vrn-A1* and *Vrn-D1* (Table 4.3). The segregation patterns in these crosses fitted the expected ratio of 15:1 for two gene models. No winter type segregants were observed for the cross between 'Taber' and TD-B, indicating that 'Taber' carries spring habit allele at *Vrn-B1*. The crosses of 'Foremost' with the three isogenic lines behaved similarly to those of 'Taber', suggesting that 'Foremost' also carries spring habit allele at *Vrn-B1*, and winter habit alleles at *Vrn-A1* and *Vrn-D1*. The crosses of 'Barrie' and 'Intrepid' with TD-B and TD-E segregated for growth habit in the F_2 generation. The segregation patterns in these crosses fitted the expected ratio of 15:1 for the complementary two gene model. However, the crosses of 'Barrie' and 'Intrepid' with TD-D did not produce winter type segregants. This indicates that 'Barrie' and 'Intrepid' carry spring habit alleles at *Vrn-A1*, and winter habit alleles at *Vrn-B1* and *Vrn-D1*.

4.4 Discussion and Conclusions

The present study demonstrated that the spring growth habit in western Canadian adapted spring wheat is mainly determined by the dominant *Vrn-A1* allele. This allele confers complete insensitivity to vernalization (Pugsley, 1971). Therefore, spring wheat

cultivars carrying the dominant *Vrn-A1* allele are usually earlier than those with only dominant alleles of *Vrn-B1* and/or *Vrn-D1* (Stelmakh, 1993). The *Vrn-A1* allele found in 83% of the Canadian spring wheat cultivars/lines examined in this study was also reported to be present in more than 50% of the spring wheat cultivars released in the United States and Argentina between 1970 and 2004 (Yan et al., 2004). This allele was also found in combination with dominant *Vrn-B1* allele in 36% of the cultivars/lines tested in the present study. The combination of dominant *Vrn-A1* and *Vrn-B1* alleles was more prevalent in cultivars/lines registered after 1996. The dominant *Vrn-B1* allele confers slight sensitivity to vernalization, resulting in delayed flowering. However, spring wheat genotypes having *Vrn-B1* in combination with the dominant *Vrn-A1* allele exhibit a vernalization insensitive phenotype due to the epistatic nature of the latter allele (Pugsley, 1971).

Genetic variation in flowering time of spring wheat cultivars with similar *Vrn* genotypes (under long day growing conditions) is attributable to earliness *per se* genes (Kato & Wada, 1999). The difference in the flowering/maturity times of the cultivars/lines having similar *Vrn* genotypes, therefore, suggests that these cultivars most likely differ in earliness *per se* genes. The spring habit allele of *Vrn-D5* was found to be present in 'Rescue' and the whole-chromosome substitution lines that involves chromosome 5D of this cultivar. This allele delays flowering as its effect is not completely masked by the spring habit allele of *Vrn-A1* (Chapter 5). The *Vrn-D5* gene has not been cloned yet in wheat, and specific markers for detecting the presence/absence of alleles of this gene are not available. Therefore, the spring habit allele of this gene may be present in some of the cultivars/lines used in this study, thereby resulting in variation in flowering/maturity time.

Considering the short growing season and lack of vernalizing conditions during growing season in western Canada (Jedel et al., 1986), vernalization insensitivity appears to be a preferred phenotype in this region. This may explain why wheat breeders in these regions have favored the vernalization insensitive dominant *Vrn-A1* allele. The early flowering/maturity of this genotype ensures timely harvest in these regions, thereby

avoiding early fall frosts that can adversely affect grain yield and quality. The vernalization sensitive dominant *Vrn-B1* allele was found singly only in four cultivars. These cultivars (AC Taber, AC Crystal, AC Foremost and AC2000) belong to the Canada Prairie Spring (CPS) wheat class that has higher yield potential compared to the Canada Western Red Spring (CWRS) class (Tames, 2005). The higher yield potential of these cultivars (in high northern growing regions) is partially related to their relative late maturity as a result of their vernalization requirement which is not fulfilled during the growing season. Vernalization response genes are known to contribute indirectly to yield by influencing flowering time (Flood & Halloran, 1986), tiller (Levy & Peterson, 1972) and spikelet number (Gororo et al., 2001; Whitechurch & Snape 2003) in sensitive genotypes. Canada Prairie Spring wheat cultivars (with vernalization sensitivity) are therefore more susceptible to early fall frost due to their late maturity, especially under late seeding conditions (Cutforth et al., 1990).

Results of this study suggest that the vernalization insensitive allele *Vrn-A1*, either singly or in combination with the dominant *Vrn-B1* allele, is more adaptive to the wheat growing regions of North America. This is due to the fact that *Vrn-A1* shortens vegetative growth period under non-vernalizing conditions (Snape et al., 2001). The dominant allele of *Vrn-D1* was not found in any of the genotypes examined, indicating that this allele has not been employed in spring wheat breeding programs in western Canada. However, a recent study concluded that *Vrn-D1* had the highest frequency among the major *Vrn* genes in the globally important CIMMYT (International Wheat and Maize Improvement Center) wheat cultivars, with the semi-dwarf Mexican variety 'Sonora 64' being one of the varieties responsible for the wide distribution of the *Vrn-D1* gene (Van Beem et al., 2005).

In this study, only the combination of dominant *Vrn-A1* and *Vrn-B1* was found in western Canadian spring wheat cultivars/lines. Stelmakh (1993) reported the combination of the dominant allele of *Vrn-A1* with either *Vrn-B1* or *Vrn-D1* to be early and high yielding in Odessa, Ukraine. The author also demonstrated that double dominant *Vrn* genotypes (*Vrn-A1 Vrn-B1 vrn-D1* and *vrn-A1 Vrn-B1 Vrn-D1*) had the highest grain

yield plant^{-1} under high temperature and drought stress conditions during grain filling. It is, therefore, recommended that different combinations of dominant *Vrn* alleles be attempted in spring wheat breeding programs in western Canada to facilitate the development of early maturing cultivars with high yield potential. Marker assisted selection will greatly facilitate the identification and incorporation of these alleles in breeding programs. Identification of earliness *per se* genes in Canadian spring wheat cultivars will further aid in modifying flowering time, especially if breeders wish to employ the vernalization sensitive dominant *Vrn* alleles in their breeding programs.

4.5 Summary

Vernalization response (*Vrn*) genes play a major role in determining the flowering/maturity times of spring-sown wheat. A representative set of 42 western Canadian adapted spring wheat cultivars/lines was characterized for their *Vrn* genes. The 42 genotypes were screened, along with 4 genotypes of known *Vrn* genes, using genome specific polymerase chain reaction primers designed for detecting the presence/absence of dominant/recessive alleles of the major *Vrn* genes: *Vrn-A1*, *Vrn-B1* and *Vrn-D1*. The dominant allele of *Vrn-A1* was present in 35 of 42 cultivars/lines. The dominant allele of *Vrn-B1* was detected in 21 cultivars/lines. Fifteen cultivars/lines had dominant alleles of *Vrn-A1* and *Vrn-B1* in combination. All cultivars/lines carried the recessive allele for *Vrn-D1*. The predominance of the dominant allele *Vrn-A1* in Canadian spring wheat appears to be due to the vernalization insensitivity of *Vrn-A1*, which confers earliness under non-vernalizing growing conditions. Wheat breeders in western Canada have incorporated the *Vrn-A1* allele into spring wheats mainly by selecting for early genotypes for a short growing season, thereby avoiding early and late season frosts. For the development of early maturing cultivars with high yield potential, different combinations of *Vrn* alleles may be incorporated into spring wheat breeding programs in western Canada.

4.6 Tables and Figures

Table 4.1. Proposed alleles at the major vernalization loci of western Canadian spring wheat cultivars/lines, inferred from polymerase chain reaction analyses.

Cultivar/Line[z]	Quality class[y]	Year of registration	Days to maturity[x]	*Vrn* locus		
				Vrn-A1	*Vrn-B1*	*Vrn-D1*
Cutler	CPS	1990	97	*Vrn-A1*	*vrn-B1*	*vrn-D1*
AC Taber	CPS	1991	108	*vrn-A1*	*Vrn-B1*	*vrn-D1*
AC Foremost	CPS	1995	104	*vrn-A1*	*Vrn-B1*	*vrn-D1*
AC Crystal	CPS	1996	103	*vrn-A1*	*Vrn-B1*	*vrn-D1*
AC Vista	CPS	1996	101	*Vrn-A1*	*vrn-B1*	*vrn-D1*
AC 2000	CPS	2000	103	*vrn-A1*	*Vrn-B1*	*vrn-D1*
Red Fife	CWRS	1885	109	*Vrn-A1*	*vrn-B1*	*vrn-D1*
Marquis	CWRS	1910	102	*Vrn-A1*	*vrn-B1*	*vrn-D1*
Early Red Fife	CWRS	1932	105	*Vrn-A1*	*Vrn-B1*	*vrn-D1*
Thatcher	CWRS	1935	99	*Vrn-A1*	*vrn-B1*	*vrn-D1*
Cadet	CWRS	1946	103	*Vrn-A1*	*vrn-B1*	*vrn-D1*
Rescue[w]	CWRS	1946	103	*vrn-A1*	*Vrn-B1*	*vrn-D1*
CR5B	CWRS	-	99	*Vrn-A1*	*Vrn-B1*	*vrn-D1*
CR5D[w]	CWRS	-	107	*Vrn-A1*	*vrn-B1*	*vrn-D1*
RC5A[w]	CWRS	-	98	*Vrn-A1*	*Vrn-B1*	*vrn-D1*
RC5B[w]	CWRS	-	106	*vrn-A1*	*vrn-B1*	*vrn-D1*
RC5D	CWRS	-	101	*vrn-A1*	*Vrn-B1*	*vrn-D1*
Saunders	CWRS	1947	97	*Vrn-A1*	*vrn-B1*	*vrn-D1*
Park	CWRS	1963	97	*Vrn-A1*	*Vrn-B1*	*vrn-D1*
Neepawa	CWRS	1969	99	*Vrn-A1*	*vrn-B1*	*vrn-D1*
Columbus	CWRS	1980	102	*Vrn-A1*	*vrn-B1*	*vrn-D1*
Katepwa	CWRS	1981	98	*Vrn-A1*	*vrn-B1*	*vrn-D1*
Roblin	CWRS	1986	99	*Vrn-A1*	*vrn-B1*	*vrn-D1*
CDC Teal	CWRS	1991	99	*Vrn-A1*	*vrn-B1*	*vrn-D1*
AC Barrie	CWRS	1994	101	*Vrn-A1*	*vrn-B1*	*vrn-D1*
AC Elsa	CWRS	1996	99	*Vrn-A1*	*vrn-B1*	*vrn-D1*
AC Splendor	CWRS	1997	97	*Vrn-A1*	*vrn-B1*	*vrn-D1*
AC Intrepid	CWRS	1997	97	*Vrn-A1*	*vrn-B1*	*vrn-D1*
McKenzie	CWRS	1997	100	*Vrn-A1*	*Vrn-B1*	*vrn-D1*
AC Abbey	CWRS	1998	99	*Vrn-A1*	*Vrn-B1*	*vrn-D1*
5600HR	CWRS	1999	101	*Vrn-A1*	*vrn-B1*	*vrn-D1*
Superb	CWRS	2001	103	*Vrn-A1*	*Vrn-B1*	*vrn-D1*
Harvest	CWRS	2002	99	*Vrn-A1*	*Vrn-B1*	*vrn-D1*
Lovitt	CWRS	2002	99	*Vrn-A1*	*Vrn-B1*	*vrn-D1*
Peace	CWRS	2002	99	*Vrn-A1*	*vrn-B1*	*vrn-D1*
5602HR	CWRS	2003	101	*Vrn-A1*	*Vrn-B1*	*vrn-D1*
CDC Go	CWRS	2004	99	*Vrn-A1*	*Vrn-B1*	*vrn-D1*
PT 213	CWRS	-	99	*Vrn-A1*	*vrn-B1*	*vrn-D1*
PT 756	CWRS	-	101	*Vrn-A1*	*Vrn-B1*	*vrn-D1*
UAW016	CWRS	-	99	*Vrn-A1*	*Vrn-B1*	*vrn-D1*
UAW024	CWRS	-	99	*Vrn-A1*	*Vrn-B1*	*vrn-D1*
Fire tail/Nemura		-	102	*Vrn-A1*	*Vrn-B1*	*vrn-D1*

[z] UAW016 and UAW024 are derived from the cross Barrie/RC5A1//McKenzie; PT 756 is Domain/Saunders; PT 213 is BW711/BW693; RC and CR are reciprocal chromosome substitution lines in the 'Cadet' and 'Rescue' backgrounds.

[y] CPS=Canada Prairie Spring; CWRS= Canada Western Red Spring. - Not registered as a cultivar.

[x] Maturity data are compiled from different sources, including data from the University of Alberta wheat breeding program and Saskatchewan Agriculture & Food (http://www.agr.gov.sk.ca/docs/crops/cereals/var2006.pdf).

[w] Also carry *Vrn-D5*.

Table 4.2. PCR markers for determining the presence of different alleles of *Vrn-A1*, *Vrn-B1* and *Vrn-D1* in hexaploid wheat (adapted from Yan et al. (2004) and Fu et al. (2005)).

Primers		Target allele(s)[x]	Exp. product size (bp)	Annealing temp. (°C)
Name[y]	Sequence (5′ to 3′)			
VRN1AF	GAAAGGAAAAATTCTGCTCG	*Vrn-A1* & *vrn-A1*	≈ 650 & 750 ≈ 500	55
VRN1R	TGCACCTTCCC(C/G)CGCCCCAT			
Intr1/B/F	CAAGTGGAACGGTTAGGACA	*Vrn-B1*	709	58
Intr1/B/R3	CTCATGCCAAAAATTGAAGATGA			
Intr1/D/F	GTTGTCTGCCTCATCAAATCC	*Vrn-D1*	1671	61
Intr1/D/R3	GGTCACTGGTGGTCTGTGC			

[y] F and R refer to the forward and reverse primers for each DNA fragment.
[x] Primers VRN1AF and VRN1R amplify DNA fragments for both dominant and recessive *Vrn-A1* alleles; The other primers amplify DNA fragment only if dominant *Vrn* allele is present.

Table 4.3. Segregation in the F_2 generations of crosses between western Canadian spring wheats and near-isogenic lines of vernalization response genes.

Cross	Number of F_2 Plants		Segregation Ratio (15:1)	
	Spring[y]	Winter[x]	χ^2	$P > \chi^2$
Barrie × TD-B	108	10	0.97	0.25
Barrie × TD-D	140	0	-	-
Barrie × TD-E	122	7	0.15	0.50
Foremost × TD-B	135	0	-	-
Foremost × TD-D	126	5	1.32	0.25
Foremost × TD-E	121	11	0.98	0.25
Intrepid × TD-B	122	11	0.93	0.25
Intrepid × TD-D	138	0	-	-
Intrepid × TD-E	115	8	0.014	0.90
Taber × TD-B	109	0	-	-
Taber × TD-D	123	11	0.87	0.25
Taber × TD-E	107	10	1.1	0.25

[y] Plants heading within 75 days after planting.
[x] Plants remaining vegetative 75 days after planting.
- No segregation for spring-winter types.
χ^2 value for significance at 5% level of probability is 3.84.

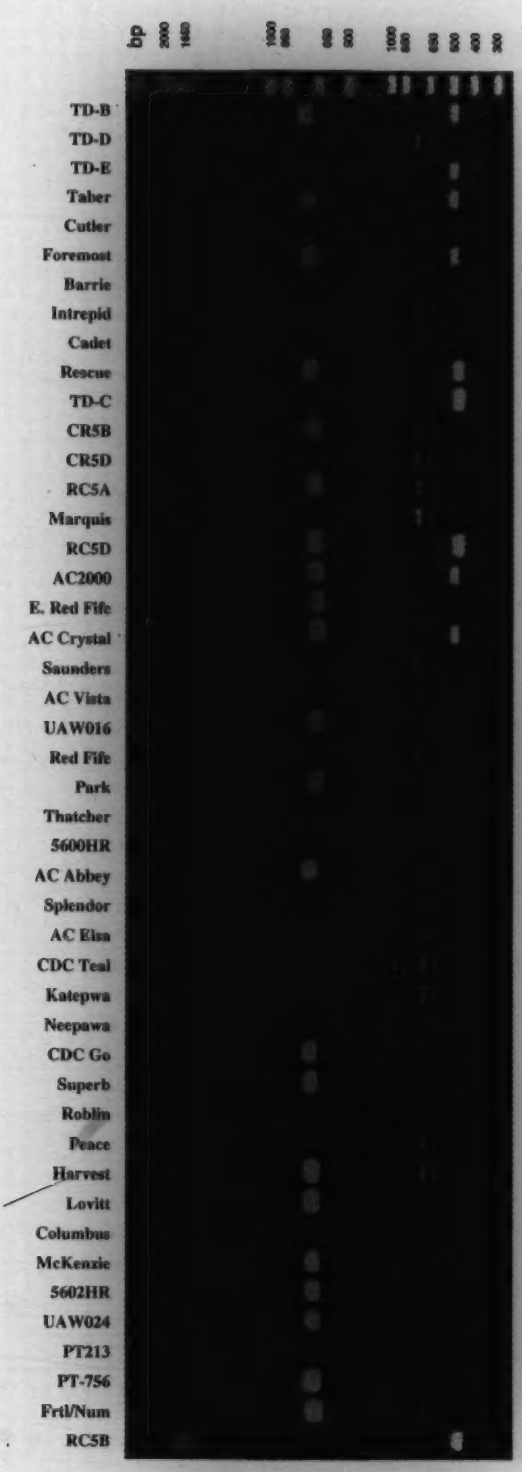


Figure 4.1. Polymerase chain reaction amplification using genome specific primers VRN1AF/VRN1R (*Vrn-A1*; bottom), Intr1/B/F//Intr1/B/R3 (*Vrn-B1*; center) and Intr1/D/F//Intr1/D/R3 (*Vrn-D1*; top), for determining the *Vrn* genotypes of 42 western Canadian spring wheat cultivars/lines.

4.7 References

Chouard, P. 1960. Vernalization and its relation to dormancy. Ann. Rev. Plant Physio. 11: 191-237.

Cutforth, H.W., C.A. Campbell, S.A. Brandt, J. Hunter, D. Judiesch, R.M. DePauw, and J.M. Clarke. 1990. Development and yield of Canadian western red spring and Canada prairie spring wheats as affected by delayed seeding in the brown and dark brown soil zones of Saskatchewan. Can. J. Plant Sci. 70: 639-660.

Flood, R.G., and G.M. Halloran. 1986. Genetics and physiology of vernalization response in wheat. Adv. Agron. 39: 87-124.

Fu, D., P. Szűcs, L. Yan, M. Helguera, J.S. Skinner, J. von Zitzewitz, P.M. Hays, and J. Dubcovsky. 2005. Large deletions within the first intron in *VRN-1* are associated with spring growth habit in barley and wheat. Mol. Genet. Genomics, 273: 54-65.

Goncharov, N.P. 2004. Response to vernalization in wheat: its quantitative or qualitative nature. Cereal Res. Commun. 32: 323-330.

Gororo, N. N., R.G. Flood, R.F. Eastwood, and H.A. Eagles. 2001. Photoperiod and vernalization responses in *T. turgidum* × *T. tauschii* synthetic hexaploid wheats. Ann. Bot. 88: 947-952.

Jedel, P.E. 1994. Inheritance of vernalization response in three populations of spring wheat. Can. J. Plant Sci. 74: 753-757.

Jedel, P.E., L.E. Evans, and R. Scarth. 1986. Vernalization responses of a selected group of spring wheat (*Triticum aestivum* L.) cultivars. Can. J. Plant Sci. 66: 1-9.

Kato, K., and T. Wada. 1999. Genetic analysis and selection experiment for narrow-sense earliness in wheat by using segregating hybrid progenies. Breed Sci. 49: 233-238.

Levy, J., and M.L. Peterson. 1972. Responses of spring wheats to vernalization and photoperiod. Crop Sci. 12: 487-490.

Major, D.J., and E.D.P. Whelan. 1985. Vernalization and photoperiod response characteristics of a reciprocal substitution series of rescue and Cadet hard red spring wheat. Can. J. Plant Sci. 65: 33-39.

Pugsley, A.T. 1971. A genetic analysis of the spring-winter habit of growth in wheat. Aust. J. Agric. Res. 22: 21-31.

Pugsley, A.T. 1972. Additional genes inhibiting winter habit in wheat. Euphytica, 21: 547-552.

Snape, J.W., K. Butterworth, E. Whitechurch, and A.J. Worland. 2001. Waiting for fine times: genetics of flowering time in wheat. Euphytica, 119: 185-190.

Stelmakh, A.F. 1993. Genetic effect of *Vrn* genes on heading date and agronomic traits in bread wheat. Euphytica, 65: 53-60.

Stelmakh, A.F. 1998. Genetic systems regulating flowering response in wheat. Euphytica, 100: 359-369.

Tames, S. 2005. Cultivar development. [Online] http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/crop1275 [07 Sep 2006].

Van Beem, J., V. Mohler, R. Lukman, M. van Ginkel, M. William, J. Crossa, and A.J. Worland. 2005. Analysis of genetic factors influencing the developmental rate of globally important CIMMYT wheat cultivars. Crop Sci. 45: 2113-2119.

Whitechurch, E. M., and J.W. Snape. 2003. Developmental responses to vernalization in wheat deletion lines for chromosomes 5A and 5D. Plant Breed. 122: 35-39.

Yan, L., M. Helguera, K. Kato, S. Fukuyama, J. Sherman, and J. Dubcovsky. 2004. Allelic variation at the *VRN-1* promoter region in polyploid wheat. Theor. Appl. Genet. 109: 1677-1686.

Chapter 5

Effects of Vernalization Genes on Earliness and Related Agronomic Traits of Spring Wheat[5]

5.1 Introduction

Despite the existence of considerable genetic variation for flowering and maturity time in western Canadian adapted spring wheat, the genetic basis of these differences is poorly understood. Environmental conditions during the growing season vary greatly with location and year, and adaptation to the various ecoregions requires cultivars of different maturity potential. The growing season in western Canada is short (95-125 days), and the development of early maturing cultivars is important to avoid frost damage that can lower both yield and quality (Tames, 2005).

The cultivation of hexaploid wheat in a wide range of environmental conditions has resulted mainly from direct selection for timing of anthesis (Gororo et al., 2001). Growth and developmental phases (tillering, stem elongation, ear emergence, anthesis and ripening) of wheat are controlled by vernalization (*Vrn*) and photoperiod response, and earliness *per se* genes (Kosner & Pankova, 1998). These genes, along with their interaction with growth temperatures (Gororo et al., 2001), play a significant role in wheat's adaptation and yield potential in many environments. Vernalization response, or high temperature inhibition of reproductive development, is widespread in temperate plant species (Flood & Halloran, 1986). Winter wheat requires exposure to a continuous cold treatment (vernalization) prior to reproductive initiation. Spring wheat generally does not have such a requirement, but some cultivars do respond to cold treatment by flowering early (Levy & Peterson, 1972; Jedel et al., 1986).

Vernalization sensitivity or insensitivity in hexaploid wheat is controlled by alleles at the major vernalization loci, *Vrn-A1*, *Vrn-B1*, *Vrn-D1* and *Vrn-D5* (Pugsley, 1971; 1972) located on the long arms of group 5 chromosomes (Law & Worland, 1997; McIntosh et al., 2003). Winter wheat possesses recessive alleles at all loci, while spring

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wheat has dominant alleles at one or more of these loci. The spring habit allele of *Vrn-A1* locus is epistatic, and confers complete insensitivity to vernalization; spring habit alleles of *Vrn-B1*, *Vrn-D1* and *Vrn-D5* confer low sensitivity to vernalization (Pugsley, 1971; 1972).

Whole-chromosome substitution lines have been used effectively in the inheritance studies of various quantitative traits of wheat (Law, 1966; Law, 1967). These lines differ only in the particular chromosome(s) substituted, facilitating the determination of the chromosomal location of genes, provided no other genes on the substituted chromosome influence the trait of interest (Kosner & Pankova, 1998). Such lines have proved useful in studying the genetics of heading time in hexaploid wheat. Halloran and Boydell (1967) determined the chromosomal location, number and nature (major or minor) of the *Vrn* genes in wheat using chromosome substitution lines. Law et al. (1976) studied the role of chromosome 5A and 5D in the genetic control of ear-emergence time of wheat using 'Chinese Spring'/'Hope' single chromosome substitution lines. Miura and Worland (1994), using chromosome substitution lines in the 'Chinese spring' background, identified genes controlling ear emergence time on homoeologous group 3 chromosomes. Kosner and Pankova (1998) used single chromosome substitution lines to detect allelic variants at the recessive *vrn* loci of winter wheat. Major and Whelan (1985) reported significant effects of the substituted chromosomes 5A, 5B and 5D carrying specific *Vrn* gene(s) on relative maturity in the greenhouse by using 'Cadet'/'Rescue' spring wheat reciprocal substitution lines. However, the effect of the particular substituted chromosome (carrying specific *Vrn* genes), in the 'Cadet'/'Rescue' substitution lines, on flowering/maturity time and other traits of agronomic importance under field conditions is not known.

An evaluation of spring wheat genotypes with different *Vrn* gene combinations under different seeding dates may improve our knowledge of the effect of these genes, not only on flowering/maturity but also on related agronomic and quality traits in northern growing regions, including western Canada. The present study was conducted to 1) investigate the effect of planting time on flowering/maturity time and important

agronomic traits in a set of chromosome substitution lines of known *Vrn* genotypes; 2) uncover the effect of specific *Vrn* genes on flowering/maturity and important agronomic traits, and 3) investigate whether some *Vrn* gene/combinations offer advantages over others in terms of maturity and grain yield in northern spring wheat growing regions.

5.2 Materials and Methods

The genetic material consisted of a set of six reciprocal whole-chromosome substitution lines of homoeologous group 5 chromosomes in two Canadian hard red spring wheat cultivar (Cadet and Rescue) backgrounds (Kosmolak et al., 1980), and eight Canadian spring wheat cultivars, 'Barrie', 'Cutler', 'Intrepid', 'Park', 'Thatcher', 'Marquis', 'Taber' and 'Foremost', representing the range of maturity in Canadian spring wheats. The chromosome substitution lines and their parents represent eight haplotypes arising from all possible allelic combinations at *Vrn-A1*, *Vrn-B1* and *Vrn-D5* loci. The *Vrn* genotypes of the chromosome substitution lines and the two parents, previously determined by Roberts and Larson (1985), were verified and those of the 8 Canadian spring wheat cultivars were determined using genome specific primers for *Vrn-A1*, *Vrn-B1* and *Vrn-D1* genes (Table 5.1). The first letter in each code of the chromosome substitution line shows the recipient parent, the second letter defines the donor parent of the specific substituted chromosome, and the last letter specifies the substituted chromosome. For example, CR5A means that chromosome 5A of Rescue has been substituted into Cadet.

Field trials were conducted at two locations in Edmonton, Alberta, Canada (53°34′N, 113°31′W) during the summers of 2004 and 2005. Soils at the experimental sites were Black Chernozems (AAFRD, 2004). Total seasonal precipitation and average temperatures for the two years are presented in Table 5.2. At each of the two locations, the 16 genotypes were planted on three seeding dates, two weeks apart in a split-plot design with four blocks. Seeding dates were assigned to main-plots and genotypes within seeding dates to subplots. Seeding dates and genotypes were replicated four times. Seeding dates were May 07, May 20 and June 03 in 2004, and May 03, May 17 and May 31 in 2005. Plot size was 6 rows, 4 m long with row spacing of 0.23m. Planting density

was 350 seeds m^{-2}. Fertilizer was applied according to soil test recommendations. During 2004, 100 kg ha^{-1} 46-0-0 (N-P_2O_5-K_2O) and 40 kg ha^{-1} 11-52-0 (N-P_2O_5-K_2O) was applied with the seed, in addition to 85 kg ha^{-1} 46-0-0 that was banded into soils at both locations in fall 2003. During 2005, 160 kg ha^{-1} 46-0-0 and 30 kg ha^{-1} 11-52-0 was applied before seeding, and 30 kg ha^{-1} 11-52-0 was applied with the seed at both locations. Weeds were controlled by the application of post emergence herbicide MCPA Amine at 1235 ml ha^{-1}.

Data were recorded on days from seeding to anthesis and physiological maturity, plant height, number of spikes m^{-2}, grains $spike^{-1}$, grain weight, grain yield and grain protein content. Time of anthesis was determined visually as the day when 75% of the heads in a plot dehisced anthers. Physiological maturity was visually determined as the number of days from seeding to when 75% of the peduncles in a plot completely lost green color. The number of calendar days from seeding to anthesis and maturity, in each year, was converted to growing degree days by summing the average daily temperatures (over a base temperature of 0°C) from the date of seeding to the date when anthesis or maturity was recorded. The number of spikes in a randomly chosen 0.5 × 2m row plot area was counted and recorded as spikes m^{-2}. Grain number $spike^{-1}$ was calculated from 10 randomly sampled heads immediately prior to harvest. Grain protein content (%) was determined using Near-Infrared Reflectance (NIR) spectroscopy using a Monochromator NIR Systems model 6500 (NIRSystems, Inc., Silver Springs, MD).

Due to the significant interaction of year with seeding date and genotype, data for individual years were analyzed separately in the MIXED procedure of SAS (SAS Institute, 2003) with location, seeding date, seeding date × location, genotype, genotype × location, genotype × seeding date, and genotype × seeding date × location considered fixed, and block (location) and block (location × seeding date) considered random. Least square means were estimated by genotypes for all seeding dates and comparisons within and between seeding dates made using an ESTIMATE statement. The genetic effects of *Vrn* genes were determined (using an ESTIMATE statement) assuming that the substituted chromosomes differ only in *Vrn* genes, and that the genetic background has

no effect. Genetic effects of *Vrn* genes were determined using pooled data from the three seeding dates. Due to the non-estimable means of the winter-type line (CR5A), maturity and yield data were used only from 1st seeding date in both years, while grain weight and grain protein data were used from 1st seeding date in 2004, and from 1st and 2nd seeding dates in 2005. In order to study the response of genotypes to seeding dates, a Shifted Multiplicative Model (SHMM) (Seyedsadr and Cornelius, 1992) with a cluster method (Crossa et al., 1993) was used following Navabi et al. (2006). For grouping the genotypes into subsets with minimum cross-over interactions, an arbitrary cut-off point was chosen at a distance of 1.5 in the dendogram.

5.3 Results

Mean seasonal temperatures did not vary greatly between the two years (Table 5.2). Mean air and soil temperatures in the week following first seeding date were low in both years. Seeding date had a significant ($P<0.05$) effect on all traits except grain weight in both years, and grain protein in 2005 (Table 5.3). Genotypes differed ($P<0.01$) for all traits in both years. Genotypes × seeding dates were also significant ($P<0.01$) for all traits, but were of lower magnitude than one or both of the main effects, as indicated by the F-values (Table 5.3). Seeding dates had a narrower range (25°C days) for days to anthesis in 2005 compared to 2004 (104°C days).

During 2004, most genotypes took the minimum degree days to anthesis and maturity when seeded earliest and the maximum degree days at the second seeding date (Table 5.4). Plant height increased for most of the genotypes with delayed seeding (data not given). Grains spike^{-1} generally decreased with delayed seeding. Grain weight did not differ with seeding date for half of the genotypes (Table 5.4). Most genotypes yielded the highest grain when seeded on the second date. Grain yields were lower when seeded earliest in 2004 which was most likely because of the delayed harvest. Cool and wet weather conditions (including snow) resulted in the delayed harvest of the first seeding date. Grain protein content of most genotypes was lowest when seeded last (Table 5.4).

During 2005, most genotypes flowered earlier in the third, and later in the first seeding date (Table 5.5). Nearly all genotypes matured earlier when planted earliest and the late when planted last. Plant height did not vary between first and second seeding date, but generally increased for the third seeding date (data not given). Grain weights were generally the greatest when seeded last. Grain yields of all genotypes differed with seeding date, exhibiting a decrease with delayed seeding (Table 5.5). Grain protein content was generally the lowest when planted latest for genotypes whose grain protein content differed with seeding date (Table 5.5).

The earliest flowering and maturing genotypes during both years were those possessing three spring habit *Vrn* alleles (RC5A), spring habit alleles *Vrn-A1* and *Vrn-B1* (Park, CR5B) or spring habit *Vrn-A1* only (Cutler, Intrepid) (Tables 5.4, 5.5). Though 'Cadet', 'Marquis' and 'Thatcher' also carry spring habit allele at *Vrn-A1*, they did not flower/mature as early as the other carriers of spring habit *Vrn-A1*. RC5A also had the highest grain protein in all three seeding dates during both years (Tables 5.4, 5.5). CR5B, besides being early flowering and maturing, was also among the top three yielding chromosome substitution lines in 2005. 'Taber' (*vrn-A1 Vrn-B1 vrn-D1*), CR5D (*Vrn-A1 vrn-B1 Vrn-D5*) and RC5B (*vrn-A1 vrn-B1 Vrn-D5*) were the latest flowering/maturing spring habit genotypes in both years. The carrier of *Vrn-A1 Vrn-D5* (CR5D) and *vrn-A1 Vrn-D5* (RC5B) had the highest grain weights among the chromosome substitution lines in both years. The cultivar 'Park', with two spring habit *Vrn* alleles, was the earliest maturing among the eight Canadian spring wheat cultivars. It also had a high grain yield and protein content, compared to cultivars of the same quality class (Barrie, Intrepid, Marquis and Thatcher) (Tables 5.4, 5.5).

The additive effects of *Vrn-A1*, *Vrn-B1* and *Vrn-D5* on days to anthesis and maturity were significant and negative in both years (Table 5.6). The negative additive effects of all three genes resulted in earlier anthesis/maturity of genotypes carrying spring habit alleles of the three genes (Table 5.6). Among the three genes, *Vrn-B1* had the greatest, while *Vrn-D5* had the least effect on accelerating anthesis/maturity in both years, except in 2004, where the effect of *Vrn-B1* on maturity was lower than that of *Vrn-*

A1 (Table 5.6). All three digenic interaction effects were significant for days to anthesis in both years, and for days to maturity in 2004. The only significant digenic interaction for days to maturity in 2005 was that of *Vrn-A1* and *Vrn-D5*. The digenic interactions were positive, thereby resulting in the inhibition of the additive effects of the three genes. The inhibitory effect was the greatest in the carrier of spring habit *Vrn-A1* and *Vrn-D5* alleles and the least in the carrier of spring habit *Vrn-A1* and *Vrn-B1* alleles (Tables 5.6, 5.7). The effects of trigenic interaction were significant and negative on days to anthesis/maturity in both years. Due to this effect, the genotype carrying spring habit alleles at three *Vrn* loci was the earliest flowering/maturing in both years (Table 5.7). Some of the genetic effects of *Vrn* genes were also significant on plant height, spike number, grain weight, grain yield and grain protein (Table 5.6).

The shifted multiplicative model with cluster method classified the 16 genotypes into 3 main groups for days to anthesis (Figures 5.1, 5.2) and maturity. The winter-type line CR5A and Taber exhibited similar responses to seeding dates in 3 of the 4 environments, where their flowering time (°C days) increased with delayed seeding. Most genotypes did not group into any discernable patterns with respect to their vernalization genes. For example, 14 of the 16 genotypes clustered together in the Michener 2004 environment (Figure 5.1). Most of these genotypes differ in vernalization response genes, but their close grouping suggests similar responses to seeding dates. Similar results were observed for the other three environments (Figures 5.1, 5.2).

5.4 Discussion and Conclusions

Although significant genotype × seeding date interactions were observed within each year and location for flowering and maturity time in the present study, these interactions did not follow a pattern which could be ascribed to the vernalization response of the genotypes. With few exceptions, the relative flowering and maturity times (°C days) or ranks of the genotypes did not change from one seeding date to another. These results suggest that vernalization requirement of spring-sown wheat is most likely not fulfilled in most years in western Canada. The presence of different *Vrn* genes did alter flowering and maturity times and related agronomic traits of the chromosome substitution

lines tested. Specific combinations of *Vrn* genes were identified in this study, which may be advantageous in northern spring wheat growing regions.

No clear pattern in terms of degree days required for flowering and maturity was observed for the three seeding dates in the present study. In terms of calendar days, the time to flowering and maturity of spring-sown wheat in western Canada generally decreases with delayed seeding (Nass et al., 1975; Baker, 1990; Cutforth et al., 1990) as was the case in this study. This pattern was not observed in terms of growing degree days in the present study. However, for the line CR5A (carrying winter habit alleles *vrn-A1*, *vrn-B1* and *vrn-D5*), the degree days required to flower increased with delayed seeding, and this pattern was more pronounced in 2004. Both vernalization sensitive and insensitive genotypes responded similarly to seeding dates, indicating that the change in flowering and maturity times of the genotypes between seeding dates was not due to the vernalization response of the genotypes. With the exception of the first seeding date in 2004, grain yield decreased with delayed seeding, which supports the results reported by Ciha (1983) and Hucl (1995), and reinforces the importance of early seeding of spring wheat in northern regions.

The ideal genetic material for examining the interaction between *Vrn* genes would have been a set of near isogenic lines of major *Vrn* genes. However, such lines are currently not available in Canadian spring wheat backgrounds and their development is cumbersome. Nevertheless, results of this study provide useful information regarding the interaction of major *Vrn* genes in wheat. Pugsley (1971, 1972) reported the spring habit *Vrn* gene at *Vrn-A1* to be epistatic to those at *Vrn-B1*, *Vrn-D1* and *Vrn-D5*. However, in this study, *Vrn-A1* was not found completely epistatic to *Vrn-B1* and *Vrn-D5* as was evident from the differences in the flowering and maturity times of chromosome substitution lines (with similar genetic background) having *Vrn-A1* but differing in either *Vrn-B1* or *Vrn-D5*. This lack of epistatic effect of *Vrn-A1*, to the best of our knowledge, has not been previously reported. The combination of *Vrn-D5* with *Vrn-A1* resulted in later flowering and maturity. The spring or winter habit allele at *Vrn-D5* is not known for the eight Canadian spring wheat cultivars. The difference in the flowering and maturity

times of these cultivars may be due to the presence of *Vrn-D5* and/or different earliness per se genes. The flowering and maturity times of genotype with spring habit alleles at *Vrn-B1* and *Vrn-D5* were not different from genotype with spring habit allele at *Vrn-B1*, suggesting that the spring habit allele of *Vrn-B1* is probably epistatic to the *Vrn-D5* allele. Shindo et al. (2003) reported that the spring habit allele of *Vrn-B1* was epistatic to the spring habit allele of *Vrn-D1*.

In the present study, specific combinations of *Vrn* genes were identified that may offer advantage in northern spring wheat growing regions. Genotypes carrying spring habit alleles at three *Vrn* loci may provide extreme earliness in regions with a very short growing season. Although grain yields of such genotypes may be comparatively lower than their late counterparts, these genotypes may be less prone to early fall frost. Genotypes with spring habit alleles at *Vrn-A1* and *Vrn-B1* appear to be a preferred *Vrn* genotype in high northern latitudes as these combine both earliness and acceptable grain yields. Genotypes carrying *Vrn-B1* singly or in combination with either *Vrn-A1* or *Vrn-D5* flower and mature comparatively earlier than the carriers of *Vrn-D5* either singly or in combination with *Vrn-A1*. The latter genotypes may have high number of grains spike^{-1} and grain weights due to their longer vegetative and grain fill period, and may yield well in regions with comparatively longer growing seasons. Such genotypes may be planted as early in the growing season as possible to avoid yield declines, which were observed in the later seeding dates in this study.

Photoperiod genes also play an important role in the flowering and maturity times of wheat. However, due to the long spring/summer days in high northern latitudes, the photoperiod requirement of sensitive spring wheat is most likely fulfilled (Klaimi and Qualset, 1973; Knott, 1986; Marshall et al., 1989) and flowering time is, therefore, not greatly affected by photoperiod response genes. Nevertheless, photoperiod sensitivity may offer yield advantage in some regions of the high northern latitudes (Dyck et al., 2004). Variation in the flowering and maturity times of the genotypes with similar *Vrn* genes in the present study suggests that these genotypes most likely differ in earliness *per se* genes. The greatest additive effects observed for *Vrn-B1* in this study may possibly be

confounded with the effects of earliness *per se* genes, as Cadet is known to have a longer basic vegetative phase than Rescue (Roberts and Larson, 1985). Earliness per se genes are generally considered to have minimal influence on developmental rate compared to photoperiod and vernalization genes. However, in the short growing season of high northern latitudes, these genes may cause flowering and maturity time variation large enough to escape drought stress during grain filling or early fall frosts in some regions. The incorporation of earliness *per se* genes is therefore necessary, along with a desirable *Vrn* gene combination in the development of early maturing cultivars for the spring wheat growing regions of high northern latitudes. The spring habit *Vrn-D1* allele was not found in 42 Canadian spring wheats (including genotypes of this study) characterized for *Vrn* genes. However, incorporation of *Vrn-D1* has been strongly recommended in spring wheats to increase grain yield and improve adaptation to late drought and heat stress (Stelmakh, 1993). It may, therefore, be useful to incorporate *Vrn-D1* either singly or in combination with other *Vrn* genes in high northern latitude spring wheats. Polymerase chain reaction markers have recently become available for the major vernalization response genes *Vrn-A1*, *Vrn-B1* and *Vrn-D1* in wheat (Yan et al., 2004; Fu et al., 2005). The use of marker-assisted selection for these genes may, therefore, help in the development of early maturing spring wheat cultivars with higher grain yield potential.

5.5 Summary

The short growing season in northern latitudes requires the growth of early maturing spring wheat (*Triticum aestivum* L.) cultivars with relatively high grain yields. This study was conducted to investigate the effect of vernalization (*Vrn*) genotype and seeding time on flowering and maturity times, and related agronomic traits of wheat. A set of reciprocal whole-chromosome substitution lines in the Cadet/Rescue hard red spring wheat background, and eight western Canadian spring wheat cultivars were examined. The 16 genotypes of known *Vrn* genes were grown over three seeding dates, two weeks apart (starting early May), at two locations in central Alberta, Canada over two years. Seeding date altered ($P<0.05$) flowering/maturity times, plant height, grain yield and grain protein, but not grain weight of all genotypes. Grain yield decreased with delayed seeding. The *Vrn* genotype carrying spring habit alleles at *Vrn-A1*, *Vrn-B1* and

Vrn-D5 flowered and matured the earliest, and had the highest grain protein content but the lowest grain yield. Genotypes with spring habit alleles *Vrn-A1* and *Vrn-B1* were early maturing and high yielding. Genotypes with spring habit *Vrn-D5* allele either singly or in combination with *Vrn-A1* were late maturing. The spring habit allele of *Vrn-A1* was not completely epistatic to *Vrn-B1* and *Vrn-D5* for flowering/maturity time. The spring habit allele of *Vrn-B1*, however, was epistatic to that of *Vrn-D5* for these traits. In northern wheat growing regions, breeding preference should be given to *Vrn* genotypes with three spring habit alleles or those with spring habit alleles of *Vrn-A1* and *Vrn-B1*. Genotypes carrying spring habit *Vrn-D5* allele singly or in combination with *Vrn-A1* should be planted as early in the growing season as possible to realize their full yield potential.

5.6 Tables and Figures

Table 5.1. Vernalization genotypes of a set of reciprocal whole chromosome substitution lines and 8 Canadian spring wheat cultivars.

Genotype	Haploid *Vrn* genotype	Reference
Cadet	*Vrn-A1 vrn-B1 vrn-D1 vrn-D5*	Roberts & Larson (1985), Chapter 4
Rescue	*vrn-A1 Vrn-B1 vrn-D1 Vrn-D5*	Roberts & Larson (1985), Chapter 4
CR5A	*vrn-A1 vrn-B1 vrn-D1 vrn-D5*	Roberts & Larson (1985), Chapter 4
CR5B	*Vrn-A1 Vrn-B1 vrn-D1 vrn-D5*	Roberts & Larson (1985), Chapter 4
CR5D	*Vrn-A1 vrn-B1 vrn-D1 Vrn-D5*	Roberts & Larson (1985), Chapter 4
RC5A	*Vrn-A1 Vrn-B1 vrn-D1 Vrn-D5*	Roberts & Larson (1985), Chapter 4
RC5B	*vrn-A1 vrn-B1 vrn-D1 Vrn-D5*	Roberts & Larson (1985), Chapter 4
RC5D	*vrn-A1 Vrn-B1 vrn-D1 vrn-D5*	Roberts & Larson (1985), Chapter 4
Marquis	*Vrn-A1 vrn-B1 vrn-D1*	Yan et al. (2004), Chapter 4
Thatcher	*Vrn-A1 vrn-B1 vrn-D1*	Yan et al. (2004), Chapter 4
Park	*Vrn-A1 Vrn-B1 vrn-D1*	Chapter 4
Barrie	*Vrn-A1 vrn-B1 vrn-D1*	Chapter 4
Cutler	*Vrn-A1 vrn-B1 vrn-D1*	Chapter 4
Foremost	*vrn-A1 Vrn-B1 vrn-D1*	Chapter 4
Intrepid	*Vrn-A1 vrn-B1 vrn-D1*	Chapter 4
Taber	*vrn-A1 Vrn-B1 vrn-D1*	Chapter 4

Allelic variation at *Vrn-D5* locus is not known for genotypes 9-16 (Marquis-Taber).

Table 5.2. Average air and soil temperatures, and total precipitation during 2004-05 at the Edmonton Research Station of University of Alberta, Alberta, Canada.

Week#	2004			2005		
	Avg. Temperature (°C)		Precipitation	Avg. Temperature (°C)		Precipitation
	Air	Soil[y]	(mm)	Air	Soil[y]	(mm)
Jan-Apr	-	-	84	-	-	92
Week 1[x]	4.6	7.8	5.3	10.3	9.5	1
Week 2	12.3	11.9	0.3	13.5	12.7	24
Week 3[x]	12.3	12.9	3.8	12.3	14.2	11.4
Week 4	13.1	13.5	29	14.6	15.2	0
Week 5[x]	14.8	16.4	10	15.3	17.3	0.3
Week 6	11.7	12.9	13	13.4	17.2	18
Week 7-10	16.8	15.5	191	16.3	18.7	29.1
Week 11-14	17.9	-	51	16.7	-	66.3
Week 15-17	14.0	-	31	13.6	-	44
Week 18	7.8	-	17	12.8	-	5.1
Week 19	9.9	-	8.9	10.4	-	9.4
Week 20	9.5	-	4.3	8.9	-	7.4
Week 21	8.6	-	0.3	7.8	-	0
Avg./Total	13.6		365	13.9		216

[y]Soil temperature at 2.5 cm depth.

[x]Weeks 1, 3 and 5 are weeks following planting of 1st, 2nd and 3rd seeding dates, respectively.

- Not applicable.

Table 5.3. Analysis of variance for 8 traits measured on 16 genotypes grown over 3 seeding dates in central Alberta, Canada during 2004-05.

Trait	Fixed Effects and their F Test Statistics					
	Seeding Date		Genotype		Seeding date × Genotype	
	2004	2005	2004	2005	2004	2005
Days to Anthesis	357***	99***	1133***	688***	46***	20***
Days to Maturity	149***	55***	140***	163***	6***	4***
Plant Height	67***	23***	267***	150***	5***	3***
Spike m^{-2}	4*	22***	7***	15***	2**	3***
Grains $Spike^{-1}$	29***	7**	21**	66***	2**	2***
Grain Weight	1^{ns}	2^{ns}	44***	59***	10***	6***
Grain Yield	17***	22***	43***	40***	17***	5***
Grain Protein	20***	4^{ns}	88***	137***	2**	8***

***,**,* Significantly different at $P<0.001$, $P<0.01$ and $P<0.05$, respectively.

^{ns} Not significant ($P \geq 0.05$).

Table 5.4. Least squares means for 5 traits of 16 genotypes planted on 3 seeding dates at 2 locations in Alberta, Canada during 2004.

	Anthesis (°C days)			Maturity (°C days)			Grain Weight (mg)			Grain Yield (t ha^{-1})			Grain Protein (%)		
Treatment	SD1	SD2	SD3	SD1	SD2	SD3	SD1	SD2	SD3	SD1	SD2	SD3	SD1	SD2	SD3
Genotype															
Cadet	855^{c}	965^{a}	930^{b}	1507^{b}	1654^{a}	1671^{a}	38.0^{b}	37.1^{b}	40.8^{a}	3.21^{b}	3.83^{a}	3.33^{b}	14.2^{a}	14.4^{a}	13.5^{b}
CR5A	1146^{c}	1314^{b}	1508^{a}	1804	-	-	34.3	-	-	3.42	-	-	13.0	-	-
CR5B	778^{c}	893^{a}	832^{b}	1445^{c}	1579^{a}	1501^{b}	34.7^{a}	34.8^{a}	35.2^{a}	2.64^{c}	3.79^{a}	3.32^{b}	14.2^{a}	14.1^{a}	13.4^{b}
CR5D	874^{c}	1011^{a}	960^{b}	1545^{b}	1704^{a}	1709^{a}	40.8^{a}	38.3^{b}	39.1^{ab}	3.23^{ab}	3.67^{a}	3.12^{b}	14.1^{a}	14.1^{a}	13.4^{b}
Rescue	829^{c}	930^{a}	886^{b}	1526^{b}	1661^{a}	1672^{a}	34.1^{a}	34.8^{a}	36.0^{a}	3.53^{a}	3.85^{a}	3.43^{a}	13.9^{a}	13.9^{a}	13.3^{b}
RC5A	755^{c}	854^{a}	795^{b}	1463^{c}	1583^{a}	1524^{b}	32.6^{c}	35.7^{b}	38.4^{a}	2.87^{b}	3.52^{a}	3.35^{a}	15.1^{a}	14.8^{a}	14.2^{b}
RC5B	874^{c}	1023^{a}	1001^{b}	1541^{b}	1724^{a}	1747^{a}	40.1^{a}	39.4^{a}	39.8^{a}	2.93^{b}	3.72^{a}	2.81^{b}	14.0^{a}	14.2^{a}	13.4^{b}
RC5D	842^{c}	936^{a}	888^{b}	1540^{b}	1627^{a}	1660^{a}	35.6^{a}	36.1^{a}	34.8^{a}	3.58^{ab}	3.84^{a}	3.27^{b}	14.1^{a}	14.0^{ab}	13.5^{b}
Barrie	826^{b}	881^{a}	866^{a}	1530^{c}	1636^{a}	1585^{b}	36.4^{ab}	34.9^{b}	37.3^{a}	2.71^{c}	3.97^{a}	3.49^{b}	14.5^{a}	13.9^{b}	13.2^{c}
Cutler	769^{c}	858^{a}	809^{b}	1448^{c}	1606^{a}	1523^{b}	33.5^{b}	33.1^{b}	36.4^{a}	3.39^{b}	4.63^{a}	3.75^{b}	14.3^{a}	13.7^{b}	13.1^{c}
Foremost	814^{c}	986^{a}	932^{b}	1560^{c}	1684^{b}	1747^{a}	37.8^{a}	33.9^{b}	37.3^{a}	3.36^{b}	4.87^{a}	3.63^{b}	12.3^{a}	11.9^{ab}	11.6^{b}
Intrepid	769^{c}	867^{a}	811^{b}	1436^{c}	1582^{a}	1523^{b}	38.1^{b}	41.2^{a}	42.2^{a}	3.26^{b}	4.26^{a}	3.96^{a}	13.9^{a}	14.1^{a}	13.7^{a}
Marquis	829^{c}	912^{a}	878^{b}	1524^{b}	1660^{a}	1668^{a}	36.9^{a}	37.7^{a}	38.6^{a}	3.37^{ab}	3.71^{a}	3.24^{b}	13.6^{ab}	13.9^{a}	13.1^{b}
Park	744^{c}	831^{a}	792^{b}	1445^{b}	1542^{a}	1480^{b}	31.7^{b}	35.8^{a}	35.2^{a}	3.20^{b}	3.96^{a}	3.55^{ab}	14.5^{a}	14.4^{a}	14.1^{a}
Taber	977^{b}	1021^{a}	1008^{a}	1644^{b}	1797^{a}	1781^{a}	40.7^{a}	32.4^{c}	35.3^{b}	3.69^{b}	4.25^{a}	2.81^{c}	12.0^{a}	11.3^{b}	10.7^{c}
Thatcher	806^{b}	878^{a}	818^{b}	1477^{b}	1587^{a}	1570^{a}	31.7^{b}	34.4^{a}	33.7^{ab}	3.34^{b}	4.32^{a}	3.38^{b}	13.9^{a}	13.6^{a}	12.7^{b}
$SE_{difference}$	9	9	9	17	17	18	1.1	1.1	1.1	0.18	0.18	0.17	0.22	0.22	0.22
S. Date[y]	843^{c}	947^{a}	920^{b}	1527^{b}	1642^{a}	1624^{a}	36.1^{a}	35.5^{a}	36.3^{a}	3.23^{b}	3.85^{a}	3.17^{b}	13.8^{a}	13.6^{a}	13.1^{b}

[x]Non-estimable due to absence of maturity.
[y]Least squares means of seeding date.
Means followed by different letters between seeding dates differ significantly at $P<0.05$.

Table 5.5. Least squares means for 5 traits of 16 genotypes planted on 3 seeding dates at 2 locations in Alberta, Canada during 2005.

	Anthesis (°C days)			Maturity (°C days)			Grain Weight (mg)			Grain Yield (t ha^{-1})			Grain Protein (%)		
Treatment	SD1	SD2	SD3	SD1	SD2	SD3	SD1	SD2	SD3	SD1	SD2	SD3	SD1	SD2	SD3
Genotype															
Cadet	903^{ab}	914^{a}	900^{b}	1579^{b}	1578^{b}	1646^{a}	36.7^{b}	37.6^{ab}	38.6^{a}	4.82^{a}	4.36^{ab}	4.27^{b}	14.1^{ab}	14.3^{a}	13.8^{b}
CR5A	1030^{c}	1048^{b}	1100^{a}	1761^{b}	1828^{a}	-	34.5^{a}	33.7^{a}	-	4.77^{a}	3.84^{b}	-	13.5^{a}	12.9^{b}	-
CR5B	861^{a}	802^{b}	792^{b}	1513^{a}	1478^{a}	1509^{a}	36.4^{a}	35.8^{a}	37.4^{a}	4.97^{a}	4.41^{b}	4.03^{b}	13.6^{a}	13.5^{a}	13.9^{a}
CR5D	946^{a}	927^{b}	937^{ab}	1642^{b}	1617^{b}	1695^{a}	38.2^{a}	38.3^{a}	39.1^{a}	4.84^{a}	4.69^{a}	4.11^{b}	14.0^{a}	14.2^{a}	13.0^{b}
Rescue	878^{a}	883^{a}	852^{b}	1536^{c}	1577^{b}	1622^{a}	33.8^{ab}	33.2^{b}	35.2^{a}	4.10^{a}	3.55^{b}	3.88^{ab}	13.7^{a}	13.9^{a}	13.2^{b}
RC5A	824^{a}	790^{b}	768^{c}	1461^{a}	1485^{a}	1498^{a}	34.1^{a}	33.2^{a}	34.8^{a}	3.71^{a}	3.30^{ab}	3.13^{b}	14.8^{a}	14.4^{b}	14.9^{a}
RC5B	953^{ab}	945^{b}	959^{a}	1601^{a}	1647^{a}	1709^{a}	38.0^{a}	38.4^{a}	38.0^{a}	4.76^{a}	4.66^{a}	3.89^{b}	14.0^{a}	14.0^{a}	12.7^{b}
RC5D	866^{ab}	877^{a}	853^{b}	1496^{c}	1549^{b}	1623^{a}	33.6^{b}	32.6^{b}	36.0^{a}	4.22^{a}	3.77^{a}	4.03^{a}	14.0^{a}	14.0^{a}	13.7^{a}
Barrie	878^{a}	812^{b}	824^{b}	1497^{b}	1516^{b}	1572^{a}	37.4^{a}	37.5^{a}	38.2^{a}	4.89^{a}	4.32^{b}	4.10^{b}	14.3^{a}	14.1^{a}	14.1^{a}
Cutler	836^{a}	787^{b}	775^{b}	1460^{b}	1463^{b}	1562^{a}	39.5^{a}	38.6^{a}	38.6^{a}	5.59^{a}	4.59^{b}	4.44^{b}	12.9^{a}	12.8^{a}	13.2^{a}
Foremost	891^{a}	892^{a}	890^{a}	1572^{c}	1643^{b}	1740^{a}	41.1^{a}	41.2^{a}	41.2^{a}	6.51^{a}	5.27^{b}	4.62^{c}	11.4^{b}	12.0^{a}	11.3^{b}
Intrepid	836^{a}	791^{b}	770^{c}	1440^{b}	1440^{b}	1482^{a}	39.9^{b}	41.4^{ab}	42.1^{a}	5.47^{a}	4.86^{b}	4.20^{c}	13.8^{a}	13.8^{a}	14.7^{a}
Marquis	889^{a}	864^{b}	839^{c}	1514^{b}	1551^{b}	1595^{a}	35.4^{ab}	35.2^{b}	37.1^{a}	4.47^{a}	3.58^{b}	3.99^{b}	13.5^{a}	13.6^{a}	13.5^{a}
Park	813^{a}	784^{b}	742^{c}	1451^{b}	1492^{a}	1523^{a}	34.0^{b}	34.5^{ab}	36.2^{a}	4.66^{a}	4.00^{b}	3.61^{b}	13.9^{b}	13.8^{b}	14.6^{a}
Taber	937^{b}	928^{b}	965^{a}	1629^{c}	1721^{b}	1768^{a}	39.6^{a}	40.9^{a}	36.6^{b}	6.51^{a}	4.89^{b}	4.13^{c}	11.4^{a}	11.5^{a}	10.9^{b}
Thatcher	845^{a}	813^{b}	821^{b}	1468^{b}	1525^{a}	1557^{a}	32.2^{b}	33.7^{ab}	34.7^{a}	4.61^{a}	4.10^{b}	3.71^{b}	13.7^{a}	13.6^{a}	13.6^{a}
$SE_{difference}$	7	7	7	17	17	17	0.9	0.9	0.9	0.20	0.20	0.19	0.20	0.20	0.20
S. Date^{y}	887^{a}	866^{b}	862^{c}	1539^{c}	1569^{b}	1607^{a}	36.5^{a}	36.6^{a}	36.9^{a}	4.93^{a}	4.26^{b}	4.00	13.5^{a}	13.5^{a}	13.4^{a}

[x]Non-estimable due to absence of maturity.

[y]Least squares means of seeding date.

Means followed by different letters between seeding dates differ significantly at $P<0.05$.

Table 5.6. Genetic effects of vernalization response genes on maturity and related agronomic traits measured on a set of chromosome substitution lines tested in Alberta, Canada during 2004-05.

Effect	Anthesis (°C days)	Maturity (°C days)	Plant height (cm)	Spikes m^{-2} (no)	Grain weight (g)	Grain yield (t ha^{-1})	Grain protein (%)
2004							
Mean	885	1504	109	789	34.4	3.85	13.9
A *vs* a	-140**	-113**	-5**	*ns*	*ns*	-0.38**	0.7**
B *vs* b	-187**	-106**	-18**	101**	-4.1**	*ns*	0.5**
D *vs* d	-91**	-55**	*ns*	*ns*	1.2*	*ns*	0.5**
A *vs* B	72**	34**	*ns*	-39**	-1.7**	-0.42**	*ns*
A *vs* D	91**	84**	1*	*ns*	*ns*	*ns*	-0.2*
B *vs* D	71**	57**	-3**	*ns*	-3**	*ns*	-0.2*
A *vs* B *vs* D	-104**	-67**	-3**	*ns*	*ns*	*ns*	0.7**
2005							
Mean	850	1539	100	490	33.7	3.59	14.1
A *vs* a	-73**	-51**	-3**	-44**	1.4**	*ns*	0.4**
B *vs* b	-127**	-144**	-17**	*ns*	-2.4**	-0.55**	*ns*
D *vs* d	-24**	-26*	*ns*	-35**	*ns*	-0.34**	0.4**
A *vs* B	11**	*ns*	*ns*	-23*	*ns*	*ns*	-0.2**
A *vs* D	27**	34**	*ns*	22*	-1.0**	-0.28**	*ns*
B *vs* D	14**	*ns*	-2**	33**	-1.8**	-0.35**	*ns*
A *vs* B *vs* D	-42**	-75**	-3**	*ns*	*ns*	-0.29**	0.6**

Additive effect of *Vrn-A1* (A *vs* a) = (+Abd – aBD – abd + ABd + AbD + ABD – abD – aBd)/4
Additive effect of *Vrn-B1* (B *vs* b) = (–Abd + aBD – abd + ABd – AbD + ABD – abD + aBd)/4
Additive effect of *Vrn-D5* (D *vs* d) = (–Abd + aBD – abd – ABd + AbD + ABD + abD – aBd)/4
Interaction effect of *Vrn-A1* and *Vrn-B1* (A *vs* B) = (–Abd – aBD + abd + ABd – AbD + ABD + abD – aBd)/4
Interaction effect of *Vrn-A1* and *Vrn-D5* (A *vs* D) = (–Abd – aBD + abd – ABd + AbD + ABD – abD + aBd)/4
Interaction effect of *Vrn-B1* and *Vrn-D5* (B *vs* D) = (+Abd + aBD + abd – ABd – AbD + ABD – abD – aBd)/4
Interaction effect of *A1*, *B1* and *D5* (A vs B *vs* D) = (+Abd – aBD – abd – ABd – AbD + ABD + abD + aBd)/4

**,* Significant at $P<0.01$ and $P<0.05$, respectively.
^{ns} Not significant ($P\geq0.05$).

Table 5.7. Least squares means for 6 traits of 8 haplotypes of *Vrn* genes grown in 3 seeding dates in Alberta, Canada during 2004-05.

Vrn haplotype	Anthesis (°C days)		Maturity (°C days)		Plant height (cm)		Grain weight (g)		Grain yield (t ha^{-1})		Grain protein (%)	
	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
vrn-A1 vrn-B1 vrn-D5	1322	1059	1804[y]	1761[y]	122	113	34.3[y]	34.1[y]	3.6[y]	4.9[y]	13.0[y]	13.2[y]
vrn-A1 vrn-B1 Vrn-D5	966	953	1671	1654	119	112	39.8	38.1	3.1	4.4	13.8	13.6
Vrn-A1 vrn-B1 Vrn-D5	948	937	1653	1651	119	111	39.4	38.5	3.3	4.5	13.8	13.7
Vrn-A1 vrn-B1 vrn-D5	917	906	1610	1601	113	107	38.6	37.6	3.4	4.4	14.0	14.1
vrn-A1 Vrn-B1 vrn-D5	888	866	1609	1557	104	94	35.5	34.1	3.5	3.9	13.9	13.9
vrn-A1 Vrn-B1 Vrn-D5	881	871	1620	1578	103	95	35.0	34.1	3.6	3.8	13.7	13.6
Vrn-A1 Vrn-B1 vrn-D5	834	818	1509	1500	100	96	34.9	36.5	3.2	4.4	13.9	13.7
Vrn-A1 Vrn-B1 Vrn-D5	801	794	1524	1483	95	90	35.6	34.0	3.2	3.3	14.7	14.7
$SE_{difference}$	6	4	11	11	1.2	1.6	0.7	0.4	0.11	0.11	0.13	0.10

[y] Data from 1st seeding date only due to absence of maturity.

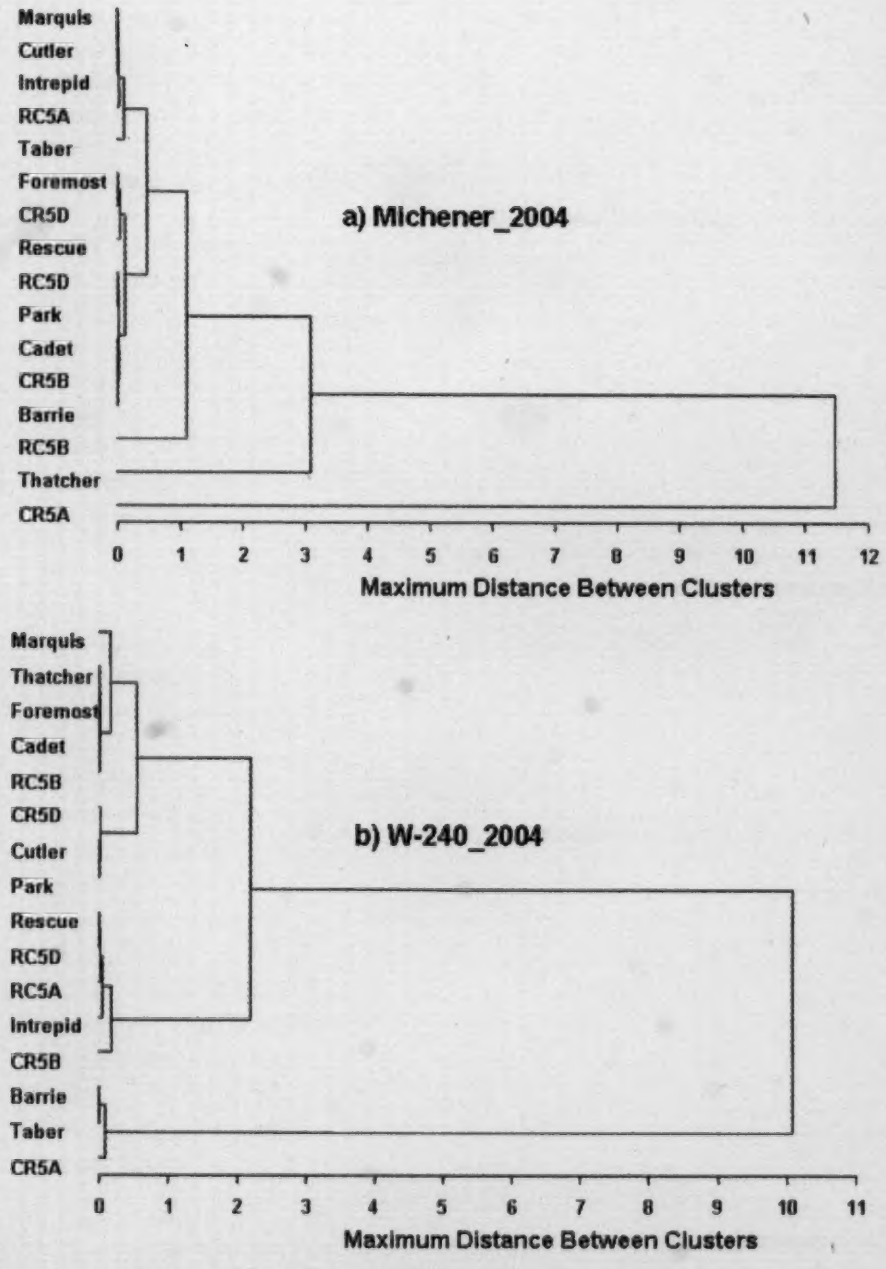

Figure 5.1. Dendograms resulting from a Shifted Multiplicative Cluster Analysis for days to anthesis of 16 genotypes grown in 3 seeding dates at two locations during 2004 at Alberta, Canada.

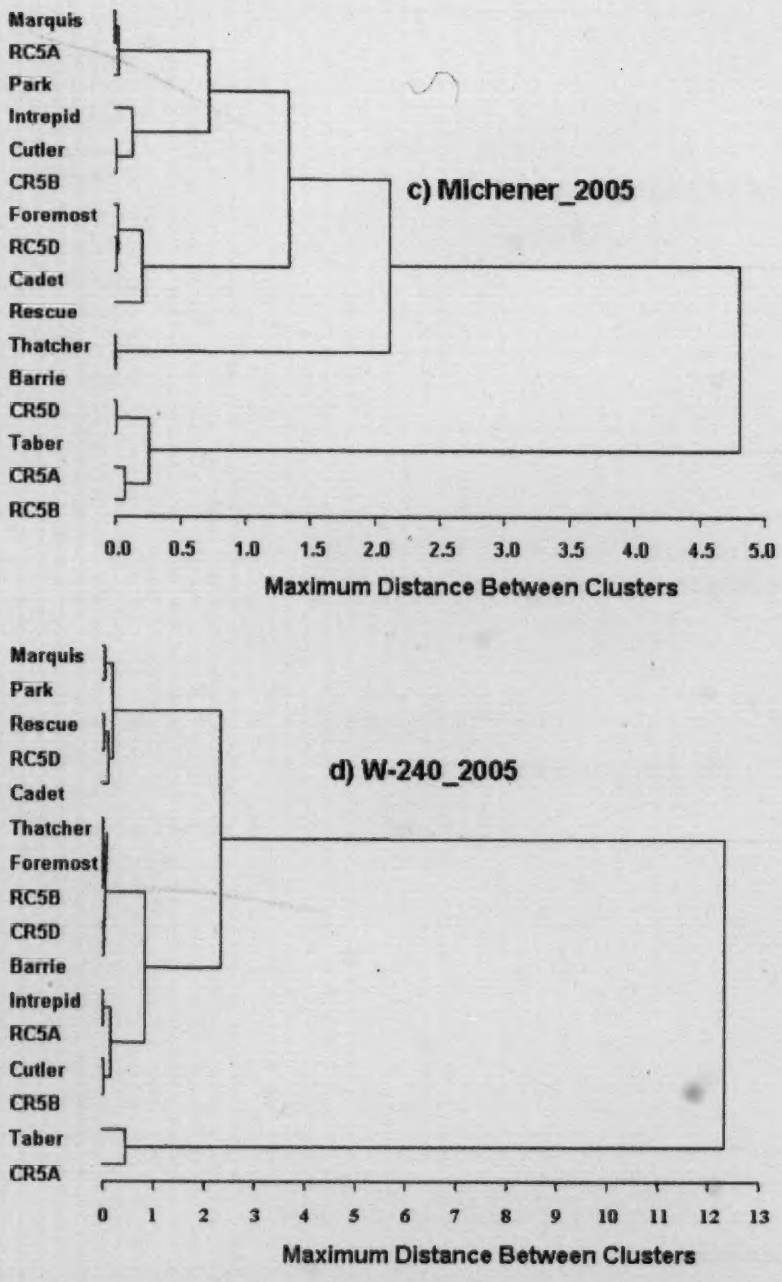

Figure 5.2. Dendograms resulting from a Shifted Multiplicative Cluster Analysis for days to anthesis of 16 genotypes grown in 3 seeding dates at two locations during 2005 at Alberta, Canada.

5.7 References

Alberta Agriculture, Food and Rural Development (AAFRD). 2004: Soil Group Map of Alberta. [Online] http://www1.agric.gov.ab.ca/soils/soils.nsf/soilgroupmap?readform. [01 Feb 06].

Baker, R.J. 1990. Agronomic performance of semi-dwarf and normal height spring wheats seeded at different dates. Can. J. Plant Sci. 70: 295-298.

Ciha, A.J. 1983. Seeding rate and seeding date effects on spring seeded small grain cultivars. Agronomy Journal, 75: 795-798.

Crossa, J., P.L. Cornelius, M. Seyedsadr, and P. Byrne. 1993. A shifted multiplicative model cluster analysis for grouping environments without genotype rank change. Theor. Appl. Genet. 85: 577-586.

Cutforth, H.W., C.A. Campbell, S.A. Brandt, J. Hunter, D. Judiesch, R.M. DePauw, and J.M. Clarke. 1990. Development and yield of Canadian western red spring and Canada prairie spring wheats as affected by delayed seeding in the brown and dark brown soil zones of Saskatchewan. Can. J. Plant Sci. 70: 639-660.

Flood, R. G., and G.M. Halloran. 1986. Genetics and physiology of vernalization response in wheat. Adv. Agron. 39: 87-124.

Fu, D., P. Szűcs, L. Yan, M. Helguera, J.S. Skinner, J. von Zitzewitz, P.M. Hays, and J. Dubcovsky. 2005. Large deletions within the first intron in *VRN-1* are associated with spring growth habit in barley and wheat. Mol. Genet. Genomics, 273: 54-65.

Gororo, N. N., R.G. Flood, R.F. Eastwood, and H.A. Eagles. 2001. Photoperiod and vernalization responses in *T. turgidum* × *T. tauschii* synthetic hexaploid wheats. Ann. Bot. 88: 947-952.

Halloran, G.M., and C.W. Boydell. 1967. Wheat chromosomes with genes for vernalization response. Can. J. Genet. Cytol. 9: 632-639.

Hucl, P. 1995. Growth response of four hard red spring wheat cultivars to date of seeding. Can. J. Plant Sci., 75: 75-80.

Jedel, P.E., L.E. Evans, and R. Scarth. 1986. Vernalization responses of a selected group of spring wheat (*Triticum aestivum* L.) cultivars. Can. J. Plant Sci. 66: 1-9.

Klaimi, Y. Y., and C.O. Qualset. 1973. Genetics of heading time in wheat (*Triticum aestivum* L.). I. The inheritance of photoperiodic response. Genetics, 74: 139-156.

Knott, D.R. 1986. Effect of genes for photoperiodism, semidwarfism, and awns on agronomic characters in a wheat cross. Crop Sci. 26: 1158-1162.

Kosmolak, F.G., R.I. Larson, and H. McKenzie. 1980. Milling and baking quality of Rescue-Cadet reciprocal substitution lines. Can. J. Plant Sci. 60: 1333-1341.

Kosner, J., and K. Pankova. 1998. The detection of allelic variants at the recessive *vrn* loci of winter wheat. Euphytica, 101:9-16.

Law, C.N. 1966. The location of genetic factors affecting a quantitative character in wheat. Genetics, 53: 487-498.

Law, C.N. 1967. The location of genetic factors controlling a number of quantitative characters in wheat. Genetics, 56: 445-461.

Law, C. N., and A.J. Worland. 1997. Genetic analysis of some flowering time and adaptive traits in wheat. New Phytologist, 137: 19-28.

Law, C.N., A.J. Worland, and B. Giorgi. 1976. The genetic control of ear emergence time by chromosomes 5A and 5D of wheat. Heredity, 36: 49-58.

Levy, J., and M.L. Peterson. 1972. Responses of spring wheats to vernalization and photoperiod. Crop Sci. 12: 487-490.

Major, D.J., and E.D.P. Whelan. 1985. Vernalization and photoperiod response characteristics of a reciprocal substitution series of rescue and Cadet hard red spring wheat. Can. J. Plant Sci. 65: 33-39.

Marshall, L., R. Busch, F. Cholick, I. Edwards, and R. Frohberg. 1989. Agronomic performance of wheat isolines diferreing for daylength response. Crop Sci. 29: 752-757.

McIntosh, R.A., K.M. Devos, J. Dubcovsky, C.F. Morris, and W.J. Rogers. 2003. Catalogue of gene symbols for wheat. [Online] http://wheat.pw.usda.gov/ggpages/wgc/2003/GeneSymbol.html. [21 April 2006].

Miura, H., and A.J. Worland. 1994. Genetic control of vernalization, day-length response, and earliness *per se* by homoeologous group-3 chromosomes in wheat. Plant Breeding, 113: 160-169.

Nass, H.G., H.W. Johnston, J.A. Macleod, and J.D.E. Sterling. 1975. Effects of seeding date, seed treatment and foliar sprays on yield and other agronomic characters of wheat, oats and barley. Can. J. Plant Sci. 55: 41-47.

Navabi, A., R-C. Yang, J. Helm, and D. Spaner. 2006. Can spring wheat-growing mega-environments in the northern Great Plains be dissected for representative locations or niche-adapted genotypes? Crop Sci. 46: 1107-1116.

Pugsley, A.T. 1971. A genetic analysis of the spring-winter habit of growth in wheat. Aust. J. Agric. Res. 22: 21-31.

Pugsley, A.T. 1972. Additional genes inhibiting winter habit in wheat. Euphytica, 21: 547-552.

Roberts, D.W.A., and R.I. Larson. 1985. Vernalization and photoperiodic responses of selected chromosome substitution lines derived from 'Rescue', 'Cadet', and 'Cypress' wheats. Can. J. Genet. Cytol. 27: 586-591.

SAS Institute. 2003: Release 9.1. SAS Institute, Inc., Cary, NC, USA.

Seyedsadr, M., and P.L. Cornelius. 1992. Shifted multiplicative models for non-additive two-way tables. Comm. Statist. B. Simul. Comp. 21: 807-832.

Shindo, C., H. Tsujimoto, and T. Sasakuma. 2003. Segregation analysis of heading traits in hexaploid wheat utilizing recombinant inbred lines. Heredity, 90: 56-63.

Stelmakh, A.F. 1993. Genetic effect of *Vrn* genes on heading date and agronomic traits in bread wheat. Euphytica, 65:53-60.

Tames, S. 2005. Cultivar development. [Online] Available: http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/crop12751 [20 April 2006].

Yan, L., M. Helguera, K. Kato, S. Fukuyama, J. Sherman, and J. Dubcovsky. 2004. Allelic variation at the *VRN-1* promoter region in polyploidy wheat. Theor. Appl. Genet. 109: 1677-1686.

Chapter 6

Genetic Relationship of Maturity Time with Grain Yield and Protein Content in Spring Wheat[6]

6.1 Introduction

High grain yield, elevated grain protein content, and early maturity are important traits in global bread wheat breeding programs. Improving these three traits simultaneously is difficult due to the negative association between grain yield and grain protein content, and the positive association between maturity and grain yield.

The negative association between grain yield and grain protein content is variable across environments and populations. Several studies report genetic correlations between grain yield and grain protein content ranging -0.37 to -0.94 (reviewed by Oury et al., 2003). Stewart and Dwyer (1990) reported an increase in grain yield following selection for this trait in spring wheat on the Canadian prairies from 1961-1982. This was accompanied with a decrease in grain protein content. Costa and Kronstad (1994) suggested that the extensive use of semidwarf cultivars with higher harvest indices may have negatively affected grain protein content in global wheat germplasm. Austin et al. (1980) suggested that grain N uptake is diluted by greater carbohydrate assimilation, resulting in lower grain protein content in high yielding semidwarf cultivars. Noaman et al. (1990), however, reported that it may be possible to develop winter wheat cultivars with high grain yield and grain protein content due to the occurrence of transgressive segregation for both traits in two winter wheat populations. Fabrizius et al. (1997) also identified progeny (in two crosses of wheat) combining the high yield and protein levels of the parents, and suggested the independent segregation of the genes controlling grain yield and grain protein content.

The positive relationship between grain yield and days to maturity poses another challenge to wheat breeders. Time to anthesis and the subsequent period from anthesis to physiological maturity (grain filling) are the main determinants of maturity in wheat

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(Duguid & Brule-Babel, 1994). The duration and rate of grain filling also determines final grain weight. A better understanding of the grain filling process and its relationship with grain weight and maturity may aid in the development of spring wheat cultivars with high yield and early maturity (Duguid & Brule-Babel, 1994). Simultaneous improvement in grain yield and earliness in wheat can be achieved either by selecting for a longer grain fill period or a faster grain fill rate (Sharma, 1994). Several studies have demonstrated no association between grain fill duration and grain yield in spring wheat (Talbert et al., 2001; Bruckner & Frohberg, 1987; Nass & Reiser, 1975). However, the rate of grain filling has been found to be positively associated with grain weight and hence grain yield (Duguid & Brule-Babel, 1994; Bruckner & Frohberg, 1987; Nass & Reiser, 1975). Przulj and Mladenov (1999) investigated the inheritance of grain fill duration in spring wheat, and found an inconsistent association between this trait and maturity. The gene actions involved in the crosses they studied were mostly additive, with some observable epistasis. There have also been reports on the positive association between grain fill duration and grain protein content in winter (Mou et al., 1994), durum (Knott & Gebeyehou, 1987) and spring (Talbert et al., 2001) wheat.

Higher grain yield, increased grain protein content, and early maturity are some of the main objectives of a typical wheat breeding program in high latitude growing regions. Simultaneous improvement of several quantitative traits requires an appropriate multiple-trait selection procedure. Smith (1936) proposed the use of a selection index: a linear function of the different traits with each trait being given a certain weight according to its importance. Several types of selection indices have been proposed (Baker, 1986). Index selection has been reported to be more efficient than single-trait selection methods in increasing aggregate genotypic worth (Gebre-Mariam & Larter, 1996; Wells & Kofoid, 1985).

The development of early maturing cultivars with high grain yield is a common objective of spring wheat breeding programs in western Canada (Duguid & Brule-Babel, 1994). The negative association between grain yield and grain protein content (along with the stringent quality requirements for cultivar registration and shorter growing season)

make it extremely difficult to achieve yield gains in the Canada Western Red Spring wheat class (Wang et al., 2002). The present study was designed to examine the relationships among grain yield, maturity and grain protein content in early maturing spring wheat germplasm. The specific objectives were to 1) investigate the genetic variation for maturity, grain yield and grain protein content in a large random population of early maturing wheat genotypes, 2) study the associations between maturity, grain yield, grain protein content and other important agronomic traits, and 3) identify genotypes not falling within the boundaries of the general interrelationship between maturity, grain yield and grain protein content.

6.2 Materials and Methods

The genetic material consisted of 130 spring wheat genotypes from the International Center for Wheat and Maize Improvement (CIMMYT) in Mexico. Genotypes, including some of the CIMMYT lines released as cultivars in different countries, in addition to inbred lines from different CIMMYT nurseries and yield trials, were selected during 1998 at Ciudad Obregon, Sonora state, Mexico. These genotypes were chosen to represent the earliest maturing lines present among germplasm grown at CIMMYT in that year. This population of 130 lines is hereafter considered as a random population representative of the early maturing spring wheat germplasm around the world. Pedigree information of some of these lines is presented in Table 6.1.

Seed increase was conducted in 1999 and 2000 in Edmonton AB Canada. Four of the ten check cultivars ('AC Splendor', 'AC Barrie', 'AC Intrepid' and 'Katepwa') were selected from the Canada Western Red Spring wheat class, with grain protein ranging from 14.1 to 15.0% and days to maturity of 108-110 days; four ('AC Taber', 'AC Foremost', 'AC Vista' and 'Cutler') from the Canada Prairie Spring wheat class, with grain protein ranging from 12 to 13% and days to maturity of 106 to 110 days; and two ('Bluesky' and 'Glenlea') from the Canada Western Extra Strong class with maturities of 108 and 110 days, respectively (AAFRD, 2005). Field trials were conducted at the Edmonton Research Station of the University of Alberta, Edmonton, AB Canada (53°34′N, 113°31′W) and at the Field Crop Development Centre, Lacombe, AB Canada

(52°28'N, 113°45'W), during 2003 and 2004. Soils at both experimental sites were Orthic Black Chernozemics (AAFRD, 2004).

The 130 experimental lines and 10 check cultivars were divided into 7 groups, each having 20 genotypes. At each of the two locations, the 140 genotypes were planted in a nested split-plot design having two replications with groups as whole plots and genotypes within groups as subplots. Plot size at Edmonton was 6 rows, 4 m long with row spacing of 0.23m. Plot size at Lacombe was 8 rows, 4m long with row spacing of 0.14m. Planting density was 350 seeds m^{-2}. Fertilizer was applied according to soil test recommendations. At Edmonton, 196 kg ha^{-1} fertilizer as 46-0-0 (N-P_2O_5-K_2O) and 117 kg ha^{-1} fertilizer as 8-24-24 (N-P_2O_5-K_2O) was broadcast after seeding during 2003. During 2004, 24 kg ha^{-1} 46-0-0 and 25 kg ha^{-1} 8-24-24 was applied with the seed in addition to 85 kg ha^{-1} 46-0-0 which was banded into the soil in fall 2003. At Lacombe, 110 kg ha^{-1} fertilizer as 6-25-30 was applied with the seed during both years (for 2003, 561 kg ha^{-1} of 5-25-30 was also banded into the soil during fall 2002). Weeds were controlled by the application of post emergence herbicides (MCPA Amine (500 ml/acre) at Edmonton and Refine/Curtail M (600 ml/acre) at Lacombe).

Data were recorded on days from seeding to anthesis and physiological maturity, plant height, number of spikes m^{-2}, grain yield, grain weight and grain protein content. Time of anthesis was determined visually as the day when 75% of the heads in a plot dehisced anthers. Physiological maturity at Edmonton was visually determined as the number of days from seeding to when 75% of the peduncles in a plot completely lost green color. Grain fill duration was calculated as the difference between days to maturity and anthesis. Grain fill rate was estimated by dividing grain yield ha^{-1} by the grain fill duration (Frederick & Bauer, 1999). Maturity data and hence grain fill duration and rate were not recorded at the Lacombe site. Harvest Index was calculated (in 2003 only) as a ratio of grain yield and above-ground biomass of a randomly sampled 1 × 2m row sample harvested just prior to grain harvest. The number of spikes in a randomly sampled 0.5 × 2m row plot area was counted and recorded as spikes m^{-2}. For estimating grain number $spike^{-1}$, number of grains m^{-2} was first estimated from the grain yield and thousand grain

weight of a plot, and then divided by spikes m^{-2}. Grain protein content (%) was determined using Near-Infrared Reflectance (NIR) spectroscopy using a Monochromator NIR Systems model 6500 (NIRSystems, Inc., Silver Springs, MD).

Data were analyzed in the MIXED procedure of SAS (SAS Institute, 2003) with all effects (Environments, Replications (environments), Groups (replications), Lines, Environments × Lines) considered random. Heterogeneity of error variance was accounted for by including the REPEATED/GROUP=ENV statement in PROC MIXED (Piepho 1999). Likelihood ratio testing was used to test if individual variance components were zero. Likelihood ratios were constructed as the difference between the -2 Residual Log Likelihood values of the reduced covariance model (without the effect being tested) and the full covariance model (with the effect being tested) (Yang, 2002). Likelihood ratio test probabilities were halved prior to comparing with Chi-square tabulated value (Self & Liang, 1987). Check cultivars were excluded for the purpose of variance and covariance components estimation. Least square means of genotypes, over the four environments, were used to determine the mean, range and standard deviation for each trait.

Broad-sense heritabilities were estimated on a plot basis as: $H = \sigma_G^2 / (\sigma_G^2 + \sigma_{GE}^2 + \sigma_e^2)$, where σ_G^2, σ_{GE}^2 and σ_e^2 are, respectively, the among-line, line × environment and error variances. The standard errors of the estimated heritabilities were computed employing the delta method (Holland et al., 2003). Selection response for 10% of the population being selected was calculated as: $R = iH\sigma_p$, where σ_p is the phenotypic standard deviation, H is the broad-sense heritability and i is the selection intensity (1.755 for 10% of the population selected (Falconer & Mackay, 1996)). Genetic and phenotypic correlations among the traits, and their standard errors, were estimated using multivariate REML implemented in the MIXED procedure (Holland, 2005). The estimated genetic ($\hat{r}_{g(xy)}$) and phenotypic ($\hat{r}_{p(xy)}$) correlations between traits x and y are given by:

$$\hat{r}_{g(xy)} = \frac{\hat{\sigma}_{G(xy)}}{\sqrt{\hat{\sigma}^2_{G(x)} \cdot \hat{\sigma}^2_{G(y)}}},$$

and

$$\hat{r}_{p(xy)} = \frac{\hat{\sigma}_{P(xy)}}{\sqrt{\hat{\sigma}^2_{P(x)} \cdot \hat{\sigma}^2_{P(y)}}} = \frac{\hat{\sigma}_{G(xy)} + \hat{\sigma}_{GE(xy)} + \hat{\sigma}_{e(xy)}}{\sqrt{\hat{\sigma}^2_{G(x)} + \hat{\sigma}^2_{GE(x)} + \hat{\sigma}^2_{e(x)}} \cdot \sqrt{\hat{\sigma}^2_{G(y)} + \hat{\sigma}^2_{GE(y)} + \hat{\sigma}^2_{e(y)}}}$$

where, $\hat{\sigma}_{G(xy)}$ and $\hat{\sigma}_{P(xy)}$ are the estimated genetic and phenotypic covariances between traits x and y; $\hat{\sigma}^2_G$ and $\hat{\sigma}^2_P$ are the estimated genetic and phenotypic variances; $\hat{\sigma}_{GE(xy)}$ and $\hat{\sigma}_{e(xy)}$ are the estimated genotype × environment and experimental error covariances between traits x and y; while $\hat{\sigma}^2_{GE}$ and $\hat{\sigma}^2_e$ are the estimated genotype × environment and experimental error variances, respectively. Correlations were considered significantly different from zero ($P<0.05$) if their approximate 95% confidence intervals did not contain zero (Holland, 2003). Confidence intervals were constructed as $r \pm z_{(0.05)}\,\sigma_e$, where r is the correlation coefficient, $z_{(0.05)}$ is the value from standardized normal distribution table at $P = 0.05$ and σ_e is the standard error of the correlation coefficients.

Selection indices for four traits were constructed as: $I = b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4$; where bs are the index coefficients and Xs are the BLUPs of genotypic values for the respective trait. The vector of Smith-Hazel index coefficients b was calculated as: $b = P^{-1}Ga$ (Baker, 1986); where P^{-1} is the inverse of the estimated phenotypic variance-covariance matrix for the four traits; G is the estimated genetic variance-covariance matrix, and a is the vector of relative economic weights of the traits included in the index. The relative economic values were assigned as reciprocals of the genotypic standard deviation of the traits (R.J. Baker, Personal communication). Two selection indices, both having protein, yield and grain fill duration in common, but differing in either maturity or anthesis, were constructed. The economic values of grain yield, protein and days to anthesis were given positive signs while those of maturity and grain fill duration were given negative signs. The expected response to selection based on index was calculated as: $R_I = iGb`/\sqrt{b`Pb}$ (Baker 1986), where $b`$ is the transposition of

b. The observed genetic gain was calculated as the difference between the mean of the selected top 10% lines and that of the entire population. The relative efficiency of index selection over direct selection for trait x was calculated as: $R_I/R_x = (b'G \cdot \sigma_{P(x)})/\sqrt{b'Pb} \cdot \sigma^2_{G(x)}$ (Baker, 1986), where $\sigma_{P(x)}$ and $\sigma^2_{G(x)}$ are the phenotypic standard deviation and genetic variance of trait x, respectively.

6.3 Results

Environmental conditions and mean grain protein content varied across locations and years (Table 6.2). Overall, grain protein content was about 2% greater in Edmonton (in both years) than Lacombe. Grain protein content at both locations was higher in 2004 than 2003 and was associated with low grain yields.

Lines differed ($P<0.01$) for all traits measured (Table 6.3). The effect of environment was significant on all measured traits, while that of line × environment was significant ($P<0.01$) for all traits except spikes m^{-2}, grains $spike^{-1}$ and harvest index. Environment effects contributed the most to the observed variation in the measured traits, followed by line effects and line × environment interaction effects. All traits exhibited a wide range of variation (Table 6.4). Maturity varied from 95 to 114 days in the experimental lines, a range 6 days greater than the check cultivars. Similar variation occurred for grain yield (2.43-5.36 t ha^{-1}) and percent grain protein (10.8-14.7%) in the experimental population. Broad-sense heritabilities across environments were low (<0.40) for grain fill duration, grain protein content, spike m^{-2}, grains $spike^{-1}$, grain fill rate, harvest index, and grain yield, and medium to high (>0.40) for grain weight, days to anthesis and maturity and plant height (Table 6.4).

For a selection intensity of 10%, the expected genetic gain (as a percent of the population mean) in the studied population was 3% for anthesis, 4% for maturity and grain fill duration, 6% for spikes m^{-2}, 8% for grain fill rate, grains $spike^{-1}$ and grain weight, 13% for plant height, 10% for grain yield and 5% for grain protein content (Table 6.4). The observed genetic gain from one cycle of selection was higher than the expected gain for all traits except plant height. The expected genetic gain from selection based on

index including maturity was 4.1 days (4% of μ) for maturity and 0.89 (7% of μ) for protein (with relative efficiencies of 114% and 127% over single trait selection for these traits). An expected decrease of 422 kg ha^{-1} in yield was observed for selection based on this index. Selection based on index including anthesis resulted in an expected change of 0.13 (1% of μ) in percent grain protein content and of 60 kg ha^{-1} (1.4% of μ) in grain yield. The expected changes in protein and yield from this index were very small, but both protein and yield did increase. Both indices had higher heritabilities (Table 6.4) than all individual traits except days to anthesis. Hence the expected genetic gain will be higher if selection is based on index than on individual traits. The index (including maturity) values were significantly correlated with maturity (-0.78), protein (0.73), yield (-0.59) and grain fill duration (-0.64), further suggesting that selection based on index values is likely to generate considerable genetic gain in each trait. The correlation between the index (including anthesis) values and individual trait values were, however, lower (0.36, 0.11, 0.08 and -0.29 with anthesis, protein, yield and grain fill duration, respectively).

Grain yield exhibited a positive genetic correlation with grains $spike^{-1}$, grain fill duration and rate, harvest index and days to anthesis and maturity (Table 6.5). Percent grain protein content was negatively correlated with grain yield, days to anthesis and maturity, grains $spike^{-1}$, and duration and rate of grain fill period. All the traits showed almost similar phenotypic associations but with lower correlation coefficients (Table 6.5). The general interrelationships of maturity, grain yield and grain protein content were also revealed when simultaneous improvement in maturity, yield and protein was attempted through index selection. Selection based on higher index values resulted in early maturity and increased percent grain protein content but decreased grain yield, while selection based on lower index values resulted in the reverse.

The cultivar 'AC Splendor' was the earliest maturing check cultivar with the highest mean grain protein content among the check cultivars (Table 6.6). 'AC Foremost' had the highest grain yield among the check cultivars. Lines identified based on their superiority over check cultivars for maturity, grain yield and/or protein content, and

based on their departure from the general interrelationships for these traits, are presented in three groups (Table 6.6). There were three lines (CIMMYT_250, 259 and 274) which matured roughly 10 days earlier than the population mean, had 2% higher grain protein content but 18-44% lower grain yield than the population mean (Table 6.6). CIMMYT_274 and CIMMYT_276 had significantly higher grain protein content than 'AC Splendor'. Group II lines had average maturity but 11-20% higher grain yield than the population mean. There were 5 lines (Group III) which roughly yielded as much as the population mean, had 0.8-2.3% greater grain protein content and were 1-6 days earlier maturing (with the exception of CIMMYT_131) than the population mean.

6.4 Discussion and Conclusions

Within a large random population of early maturing spring wheat lines grown in a high latitude environment, a strong positive association between maturity and grain yield, a strong negative association between maturity and grain protein content, and a strong negative association between grain yield and grain protein content was observed. The negative association of grain protein content with maturity suggests that one important objective of spring wheat breeding programs in high northern latitudes (early maturity and high grain protein content) can be readily achieved simultaneously. However, simultaneous improvements in grain protein and grain yield, and early maturity and grain yield will be extremely difficult, due to the strong negative associations of these traits.

Despite the negative association between grain yield and grain protein content in the present study, some lines departed from the general interrelationships of maturity, yield and protein content. Lines with average grain yield but significantly higher grain protein than the population mean were identified (CIMMYT_131, 248 and 276). Similarly, lines with average maturity and significantly higher grain yield than the population mean were also identified (CIMMYT_077, 151 and 217). The existence of such lines, and the considerable genetic variability exhibited for maturity, grain yield and grain protein content, suggests the possibility of simultaneously improving the three traits. The presence of such outlying genotypes has also been reported previously. Fabrizius et al. (1997) identified progeny combining high grain yield and protein in two

crosses of wheat. Costa and Kronstad (1994) reported moderate correlations between grain yield and protein, and concluded that these two traits could be improved simultaneously. Mesfin et al. (2000) did not find any association of grain protein with grain yield, days to heading and plant height in two hard red spring wheat populations, and concluded that they could select lines with high protein and acceptable yield, but with later maturity and taller plants. Loffler et al. (1985) identified hard red spring wheat genotypes close to the population mean for grain protein, but significantly higher than the mean for grain yield.

The high positive genetic and phenotypic associations between grain yield and grain fill rate and grains spike^{-1} indicate that the latter two traits contribute more than grain fill duration and grain weight to high yield potential. Grain number spike^{-1} was the major contributing factor to increased grain yield, as was also reported by Slafer and Andrade (1993), Siddique et al. (1989), Austin et al. (19[illegible]) and Hucl and Baker (1987). The negative association between harvest index and gra[illegible]otein content observed in this study has been previously reported (Costa & Kronstad, 1994; Loffler & Busch, 1982; Kramer, 1979). Extensive use of semidwarf cultivars as a means to increase grain yield has been suggested to be the cause of this negative relationship (Costa & Kronstad, 1994; Austin et al., 1980).

Based on the positive association between grain fill duration and grain protein content, Talbert et al. (2001) suggested that selection for early heading and longer grain fill duration may help circumvent the undesirable negative association between grain yield and grain protein content. In the present study, however, grain fill duration and grain protein content were negatively correlated. By accounting for the associations of grain protein content with days to anthesis and grain fill duration, and of grain fill duration with grain yield, it may be more appropriate to select for delayed flowering and shorter grain fill duration. As grain protein content and days to anthesis were moderately correlated in the present study, delayed flowering may not be associated with lower grain protein accumulation. Indeed, delayed flowering may have a positive effect on grain protein content if accompanied with shorter grain fill duration. Furthermore, as the

association between grain fill duration and grain yield was also moderate, the decrease in grain yield due to shorter grain fill duration may be only moderate. If the delayed flowering involves longer spike growth period, then the increased number of spikelets spike^{-1} and high percentage of fertile florets (as a result of greater partitioning of assimilates towards spike compared to that of stem) is expected to increase grain yield (Gonzalez et al., 2003; Slafer et al., 2001; Siddique et al., 1989). A longer vegetative period (planting to anthesis) may also serve to increase grain yield through increased grains spike^{-1} and grain weight, as reported by Gebeyehou et al. (1982) in durum wheat and Bingham (1969) in bread wheat.

The positive genetic association between maturity and grain fill duration, in the present study, was much higher than that between anthesis and grain fill duration. This, coupled with the observation that grain yield had stronger association with grain fill rate compared to that with grain fill duration, again suggests that selecting for a longer spike growth period and a shorter grain fill duration with higher grain fill rate may aid in selecting for early maturity and high grain yield. This is supported by the fact that genetic yield gains in wheat over time have been generally attributed to increased numbers of grains spike^{-1} rather than grain weight (Slafer & Andrade, 1993; Siddique et al., 1989; Austin et al., 1989; Hucl & Baker, 1987).

Results of the present study suggest the possibility of developing early maturing spring wheat without negatively affecting grain yield and grain protein content, through selection for shorter grain fill duration and faster grain fill rate. However, a point of concern is the low heritabilities of rate and duration of grain fill, implying that genetic gain in these traits would require multi-environment testing. The problem of low heritability of grain fill duration may be solved by indirect selection for shorter grain fill duration through delayed anthesis and early maturity, both exhibiting high heritabilities. To better understand the processes leading to the departure from the normal interrelationships of maturity, grain yield and grain protein, the outlying genotypes identified in this study should be further examined.

6.5 Summary

High grain yield and grain protein content, and early maturity are important traits in global bread wheat (*Triticum aestivum* L.) breeding programs. Improving these three traits simultaneously is difficult due to the negative association between grain yield and grain protein content and the positive association between maturity and grain yield. The genetic relationship between maturity, grain yield and grain protein content was investigated in a population of 130 early maturing spring wheat lines in a high latitude (52 to 53°N) wheat growing region of Canada. Grain protein content exhibited negative genetic correlation with maturity (-0.87), grain fill duration (-0.78), grain fill rate (-0.49), grain yield (-0.93) and harvest index (-0.71). Grain yield exhibited positive genetic correlation with maturity (0.69), rate (0.78) and duration (0.49) of grain fill, and harvest index (0.55). Despite the positive association between maturity and grain yield, and negative association between grain yield and grain protein content, higher yielding lines with medium maturity and higher grain protein content were identified. Broad-sense heritabilities were low (<0.40) for rate and duration of grain fill, grain protein content, spike m^{-2}, grains $spike^{-1}$, harvest index, and grain yield, and medium to high (>0.40) for grain weight, days to anthesis and maturity, and plant height. Selection for longer pre-anthesis and shorter grain fill periods may help circumvent the negative association between grain yield and grain protein content. Selection for shorter grain fill periods and higher grain fill rate may be a useful strategy for developing early maturing cultivars with acceptable grain yields in northern wheat growing regions.

6.6 Tables

Table 6.1. Pedigrees/Names of selected CIMMYT lines/cultivars grown in four environments in Alberta, Canada during 2003-04.

Line #	Pedigree/Name
CIMMYT002	TOBARITO M 97
CIMMYT077	SERI M 82
CIMMYT090	PASTOR
CIMMYT117	ODK16/PDGA//AU/JTS179/3/NAC/4/OPATA/5/CNO79/PRL
CIMMYT131	PRL/VEE#6//VEE/MYNA
CIMMYT151	CEP8927
CIMMYT158	KAUZ*2//K134(60)/VEE
CIMMYT217	CHEN/AEGILOPS SQUARROSA (TAUS)//BCN
CIMMYT248	BOW/PRL//BUC/3/LUAN
CIMMYT250	RABE/PARUS//PARUS
CIMMYT259	WEAVER/ROBLIN
CIMMYT274	CMH73A.497/2*CNO79//CMH76.173/CNO79
CIMMYT276	CMH81.38/2*KAUZCAL/NH//H567.71/3/2*NING 7840/CMH83.2277/5/BOW/2*NING 7840/4/CMH83.227
CIMMYT304	Not available
CIMMYT312	"NG8319//SHA4/LIRA"

Table 6.2. Total seasonal (May-August) rainfall, mean seasonal temperatures, and mean values of maturity, yield and protein of 140 spring wheat genotypes grown in four environments in Alberta, Canada during 2003-04.

Location	Year	Rainfall (mm)	Temperature (°C)	Maturity (days)	Grain Yield (t ha^{-1})	Grain Protein (%)
Edmonton	2003	186	15.6	101	5.86	13.0
Edmonton	2004	328	14.3	111	4.73	13.9
Lacombe	2003	131	14.9	-	4.79	11.2
Lacombe	2004	250	13.4	-	1.93	11.7
S. Error				0.4	0.19	0.2

- Data not recorded.

Table 6.3. Variance component estimates for 11 traits measured on a population of 130 spring wheat lines grown in four environments in Alberta, Canada during 2003-04.

Trait	Variance Components				
	Environment	Rep(Env)	Groups (Rep)	Line	Env*Line
Days to Anthesis	32**	0.02	0.04**	2.5**	0.8**
Spikes m^{-2}	33506**	298**	138	1363**	236
Grains $Spike^{-1}$	277.4**	2.5	0.09	2.64**	0
Plant Height	18.2*	1.62**	1.89**	48.4**	3.82**
Grain Fill Rate	143.8**	0**	75.3**	90.1**	97.3**
Grain Fill Duration	13.5**	0.02	0.16	3.4**	2.7**
Days to Maturity	48.3**	0.06	0.16*	8.7**	3.2**
Grain Weight	11.2**	0	0.09	7.95**	2.25**
Harvest Index	0.001*	0.00*	0.00	0.001**	0.00
Grain Yield	1.33*	0.02*	0.09**	0.11**	0**
Grain Protein	1.6**	0	0.1**	0.81**	0.20**

**, * Significantly different from zero at $p<0.01$ and $p<0.05$, respectively, based on likelihood ratio test.

Table 6.4. Means, standard deviations (SD), ranges, heritabilities and selection responses (R) for 11 traits measured on a population of 130 spring wheat lines and 10 check cultivars grown in four environments in Alberta, Canada during 2003-04.

Trait		Mean	SD	Min.	Max.	Heritability	R_e	R_o
Days to Anthesis	Lines	67	1.7	63	70	0.63 (0.04)[y]	2.3	2.7
	Checks	66	1.4	64	68	-	-	-
Spikes m^{-2} (no)	Lines	490	62	380	720	0.14 (0.03)	28	61
	Checks	530	88	400	660	-	-	-
Grains $Spike^{-1}$ (no)	Lines	27	5	15	39	0.14 (0.03)	2.2	4.6
	Checks	25	4	19	31	-	-	-
Plant Height (cm)	Lines	79	7	66	106	0.71 (0.03)	10	9.6
	Checks	91	10	76	105	-	-	-
Grain Fill Rate	Lines	129	15	82	162	0.32 (0.06)	11	13
($kg\ ha^{-1}\ day^{-1}$)	Checks	133	8	121	147	-	-	-
Grain Fill Duration	Lines	42	2	34	48	0.29 (0.04)	1.5	2.2
(days)	Checks	40	3	36	44	-	-	-
Days to Maturity	Lines	106	3.3	95	114	0.54 (0.04)	3.6	5.2
	Checks	103	4.6	97	110	-	-	-
Grain Weight (mg)	Lines	41	2.9	32	49	0.49 (0.04)	3.4	4.5
	Checks	41	3.3	36	46	-	-	-
Harvest Index	Lines	0.50	0.04	0.39	0.57	0.35 (0.05)	0.03	0.04
	Checks	0.48	0.04	0.44	0.55	-	-	-
Grain Yield ($t\ ha^{-1}$)	Lines	4.33	0.46	2.43	5.36	0.38 (0.04)	0.45	0.55
	Checks	4.31	0.36	3.56	4.77	-	-	-
Grain Protein (%)	Lines	12.4	0.8	10.8	14.7	0.29 (0.04)	0.7	1.2
	Checks	12.3	0.8	11.0	13.3	-	-	-
Index (maturity)		-29.9	2.3	-34.6	-22.8	0.60 (0.04)	2.2	-
Index (anthesis)		-17.5	1.4	-20.4	-13.0	0.63 (0.04)	1.5	-

R_e= Expected response from 10% selection.
R_o= Observed response from 10% selection.
[y] Standard error of the heritability estimate.
- Not estimated.

Table 6.5. Genetic (G) and Phenotypic (P) correlation coefficients for 11 traits measured on a population of 130 early maturing spring wheat lines grown in four environments in Alberta, Canada during 2003-04.

Trait	Coef.	Spikes m^{-2}	Grains $Spike^{-1}$	Plant Height	Grain Fill Rate	Grain Fill Duration	Maturity	Grain Weight	Harvest Index	Grain Yield	Grain Protein
Anthesis	G	-0.42*	0.63*	0.03	0.63*	0.39*	0.82*	0.07	0.08	0.66*	-0.56*
	P	-0.22*	0.24*	0.10	0.38*	0.04	0.60*	0.12*	0.14*	0.41*	-0.21*
Spikes m^{-2}	G		-0.79*	-0.28*	-0.37	-0.19*	-0.44*	-0.32*	-0.02	-0.44*	0.49*
	P		-0.61*	-0.18*	-0.32	0.09*	-0.09	-0.16*	-0.10*	-0.18*	0.13*
Grains $Spike^{-1}$	G			0.10	0.52*	0.34*	0.68*	-0.22	0.20	0.80*	-0.93*
	P			0.07	0.59*	0.06	0.21*	-0.12*	0.16*	0.39*	-0.19*
Plant Height	G				0.35*	-0.33*	-0.[illegible]	0.11	-0.64*	0.01	0.08
	P				0.32*	-0.25*	-0.[illegible]	0.13*	-0.31*	0.17*	-0.02
Grain Fill Rate	G					-0.14	0.47*	0.24	0.14	0.78*	-0.49*
	P					-0.37*	0.00	0.19*	0.16*	0.84*	-0.18*
Grain Fill Duration	G						0.70*	0.17	0.63*	0.49*	-0.78*
	P						0.75*	0.01	0.26*	0.12*	-0.20*
Maturity	G							0.15	0.45*	0.69*	-0.87*
	P							0.11	0.25*	0.45*	-0.31*
Grain Weight	G								0.21	0.25*	-0.09
	P								0.10	0.22*	-0.06
Harvest Index	G									0.55*	-0.71*
	P									0.33*	-0.28*
Grain Yield	G										-0.93*
	P										-0.34*

*Significantly different from zero ($P < 0.05$).
No asterisk indicates non-significant estimate.

Table 6.6. Least square means for 8 traits of selected[y] spring wheat lines and check cultivars grown in four environments in Alberta, Canada during 2003-04.

Genotype	Grains Spike^{-1} (no)	GFR[x] (kg ha^{-1} day^{-1})	GFD[x] (days)	Maturity (days)	Grain Weight (mg)	HI[x]	Grain Yield (t ha^{-1})	Grain Protein (%)
Experimental Lines								
Mean	27	129	42	106	41	0.50	4.33	12.4
Group I (Early maturity or high yield potential)								
CIMMYT090	30	152	43	110	41	0.52	5.36	10.8
CIMMYT250	19	114	37	95	42	0.45	3.50	14.0
CIMMYT259	22	122	36	95	41	0.44	3.56	13.8
CIMMYT274	15	82	40	98	38	0.40	2.43	14.6
Group II (Average maturity with high grain yield potential)								
CIMMYT002	33	153	39	103	37	0.52	4.80	12.1
CIMMYT077	26	147	42	107	39	0.53	5.20	11.9
CIMMYT117	26	133	42	105	46	0.54	4.81	12.6
CIMMYT151	27	162	41	107	40	0.47	5.05	11.3
CIMMYT217	23	137	43	106	46	0.54	4.90	11.6
CIMMYT304	31	141	40	106	38	0.54	4.81	11.9
Group III (High grain protein potential with average yield potential)								
CIMMYT131	22	117	44	109	44	0.50	4.29	13.7
CIMMYT158	26	128	41	102	43	0.51	4.34	13.2
CIMMYT248	25	157	39	105	45	0.51	4.51	13.7
CIMMYT276	24	148	37	101	39	0.45	4.06	14.7
CIMMYT312	27	140	37	100	41	0.50	4.15	13.3
Check Cultivars								
Mean	25	133	40	104	41	0.48	4.31	12.3
AC Splendor	24	121	36	97	36	0.44	3.56	13.3
AC Foremost	26	129	44	109	42	0.55	4.77	11.5
Cutler	26	147	40	101	41	0.51	4.41	12.5
S. Error (diff.)	4	14	2	2	2	0.03	0.31	0.6

[y] Selected based on maturity, grain yield and protein.

[x] GFD= Grain fill duration, GFR= Grain fill rate, HI= Harvest index.

6.7 References

Alberta Agriculture, Food and Rural Development (AAFRD). 2004. Soil Group Map of Alberta. [Online] http://www1.agric.gov.ab.ca/soils/soils.nsf/soilgroupmap?readform. [26 Sep 2005].

Alberta Agriculture, Food and Rural Development (AAFRD). 2005. Cereal and oilseed yield and performance data. [Online] http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/crop80?opendocument. [26 Sep 2005].

Austin, R.B., M.A. Ford, and C.L. Morgan. 1989. Genetic improvement in the yield of wheat: A further evaluation. J. Agric. Sci. 112: 295-301.

Austin, R.B., J. Bingham, R.D. Blackwell, L.T. Evans, M.A. Ford, C.L. Morgan, and M. Taylor. 1980. Genetic improvements in winter wheat yields since 1900 and associated physiological changes. J. Agric. Sci. Camb. 94: 675-689.

Baker, R.J. 1986. Selection indices in plant breeding. CRC Press, Boca Raton, Florida.

Bingham, J. 1969. The physiological determinants of grain yield in cereals. Agric. Prog. 44: 30-42.

Bruckner, P.L., and R.C. Frohberg. 1987. Rate and duration of grain fill in spring wheat. Crop Sci. 27: 451-455.

Costa, J.M., and W.E. Kronstad. 1994. Association of grain protein concentration and selected traits in hard red winter wheat populations in the Pacific Northwest. Crop Sci. 34: 1234-1239.

Duguid, S. D., and A. L. Brule-Babel. 1994. Rate and duration of grain filling in five spring wheat (*Triticum aestivum* L.) genotypes. Can. J. Plant Sci. 74: 681-686.

Fabrizius, M.A., M. Cooper, and K.E. Basford. 1997. Genetic analysis of variation for grain yield and protein concentration in two wheat crosses. Aus. J. Agric. Res. 48: 605-614.

Falconer, D.S., and T.F.C. Mackay. 1996. Introduction to quantitative genetics. Fourth Edition. Prentice Hall, Harlow, London. p. 379.

Frederick, J.R., and P.J. Bauer. 1999. Physiological and numerical components of wheat yield. In: E.H. Satorre and G.A. Slafer (eds.), Wheat, ecology and physiology of yield determination. Haworth Press, NY. p. 55.

Gebeyehou, G., D.R. Knott, and R.J. Baker. 1982. Relationships among durations of vegetative and grain filling phases, yield components, and grain yield in durum wheat cultivars. Crop Sci. 22: 287-290.

Gebre-Mariam, H., and E.N. Larter. 1996. Genetic response to index selection for grain yield, kernel weight and per cent protein in four wheat crosses. Plant Breed. 115: 459-464.

Gonzalez, F.G., G.A. Slafer, and D.J. Miralles. 2003. Floret development and spike growth as affected by photoperiod during stem elongation in wheat. Field Crops Res. 81: 29-38.

Holland, J.B. 2006. Estimating genotypic correlations and their standard errors using multivariate restricted maximum likelihood estimation with SAS Proc MIXED. Crop Sci. 46: 642-654.

Holland, J.B., W.E. Nyquist and C.T. Cervantes-Martinez. 2003. Estimating and interpreting heritability for plant breeding: An update. Plant Breed. Rev. 22: 9-111.

Hucl, P., and R.J. Baker. 1987. A study of ancestral and modern Canadian spring wheats. Can. J. Plant Sci. 67: 87-97.

Knott, D.R., and G. Gebeyehou. 1987. Relationships between the lengths of the vegetative and grain filling periods and agronomic characters in three durum wheat crosses. Crop Sci. 27: 857-860.

Kramer, T.H. 1979. Environmental and genetic variation for protein content in winter wheat (*Triticum aestivum* L.). Euphytica, 28: 209-218.

Loffler, C.M., and R.H. Busch. 1982. Selection for grain protein, grain yield, and nitrogen partitioning efficiency in hard red spring wheat. Crop Sci. 22: 591-595.

Loffler, C.M., T.L. Rauch, and R.H. Busch. 1985. Grain and plant protein relationships in hard red spring wheat. Crop Sci. 25: 521-524.

Mesfin, A., R.C. Frohberg, K. Khan, and T.C. Olson. 2000. Increased grain protein content and its association with agronomic and end-use quality in two hard red spring wheat populations derived from *Triticum tugidum* L. var. *dicoccoides*. Euphytica, 116: 237-242.

Mou, B., W.E. Kronstad, and N.N. Saulescu. 1994. Grain filling parameters and protein content in selected winter wheat populations: II. Associations. Crop Sci. 34: 838-841.

Nass, H.G., and B. Reiser. 1975. Grain filling period and grain yield relationships in spring wheat. Can. J. Plant Sci. 55: 673-678.

Noaman, M.M., G.A. Taylor, and J.M. Martin. 1990. Indirect selection for grain protein and grain yield in winter wheat. Euphytica, 47: 121-130.

Oury, F.X., P. Berard, M. Brancourt-Hulmel, C. Depatureaux, G. Doussinault, N. Galic, A. Giraud, E. Heumez, C. Lecomte, P. Pluchard, B. Rolland, M. Rousset, and M. Trottet. 2003. Yield and grain protein concentration in bread wheat: a review and a study of multi-annual data from a French breeding program. J. Genet. Breed. 57: 59-68.

Piepho, H-P. 1999. Stability analysis using the SAS system. Agron. J. 91: 154-160.

Przulj, N., and N. Mladenov. 1999. Inheritance of grain filling duration in spring wheat. Plant Breed. 118: 517-521.

SAS Institute. 2003. Release 9.1. SAS Institute, Inc., Cary, NC, USA.

Self, S.G., and K-Y. Liang. 1987. Asymptotic properties of maximum likelihood estimators and likelihood ratio tests under nonstandard conditions. J. Am. Stat. Assoc. 82: 605-610.

Sharma, R.C. 1994. Early generation selection for grain-filling period in wheat. Crop Sci. 34: 945-948.

Siddique, K.H.M., R.K. Belford, M.W. Perry, and D. Tennant. 1989. Growth, development and light interception of old and modern wheat cultivars in a Mediterranean-type environment. Aus. J. Agric. Res. 40: 473-487.

Slafer, G.A., L.G. Abeledo, D.J. Miralles, F.G. Gonzalez, and E.M. Whitechurch. 2001. Photoperiod sensitivity during stem elongation phase as an avenue to raise potential yield in wheat. Euphytica, 119: 191-197.

Slafer, G.A., and F.H. Andrade. 1993. Physiological attributes related to the generation of grain yield in bread wheat cultivars released in different eras. Field Crops Res. 31: 351-367.

Smith, H.F. 1936. A discriminant function for plant selection. Ann. Eugen. (London) 7: 240-250.

Stewart, D.W., and L.M. Dwyer. 1990. Yields and protein trends of spring wheat (*Triticum aestivum* L.) on the Canadian prairies, 1961-1982. Can. J. Plant Sci. 70: 33-44.

Talbert, L.E., S.P. Lanning, R.L. Murphy, and J.M. Martin. 2001. Grain fill duration in twelve hard red spring wheat crosses: Genetic variation and association with other agronomic traits. Crop Sci. 41: 1390-1395.

Wang, H., T.N. McCaig, R.M. DePauw, F.R. Clarke, and J.M. Clarke. 2002. Physiological characteristics of recent Canada western red spring wheat cultivars: Yield components and dry matter production. Can. J. Plant Sci. 82: 299-306.

Wells, W.C., and K.D. Kofoid. 1985. Selection indices to improve an intermating population of spring wheat. Crop Sci. 26: 1104-1109.

Yang, R-C. 2002. Likelihood-based analysis of genotype-environment interactions. Crop Sci. 42: 1434-1440.

Chapter 7

Summary, General Discussion and Conclusions

7.1 Introduction

Early maturity is one of the important objectives in spring wheat breeding programs globally. Earliness ensures timely crop harvest and may also protect wheat from biotic and abiotic stresses such as disease, heat and drought (Poehlman & Sleper, 1995). The high latitude wheat growing regions of the northern hemisphere (e.g. western Canada) are characterized by short growing seasons, low temperatures early and late in the growing season, and long days (>14 hours) during the growing season. Due to the short growing season (95-125 days) in western Canada, the development of early maturing cultivars is important to avoid frost damage and lessen the probability of pre-harvest sprouting in years of cold and wet harvest conditions.

Flowering time of wheat is determined by three component traits, photoperiod sensitivity, vernalization requirement and earliness *per se* (Kato and Yamagata, 1988). Photoperiod sensitivity is the requirement of a certain period of long day to initiate flowering. Vernalization requirement is the requirement of exposure to cold temperatures to initiate or accelerate flowering. Earliness *per se* is the difference in developmental rate, independent of day length and vernalization response. Genes of these three systems, along with their interaction with growth temperatures (Gororo et al., 2001), play a significant role in wheat's adaptation and yield potential in many environments.

Vernalization response genes play a major role in the flowering time of spring wheat grown in northern growing regions. Stelmakh (1993, 1998) reported that *Vrn* genes have differential effects on heading time, plant height and yield components. Genotypes having two dominant alleles in combination at two vernalization loci tend to mature early and exhibit higher yield potential. Triple dominant genotypes were reported to be early but low yielding. This suggests the possibility of combining specific dominant *Vrn* alleles in spring wheat cultivars to improve grain yield potential while maintaining their earliness. The negative association between grain yield and grain protein content (along

with the stringent quality requirements for cultivar registration and shorter growing season) make it extremely difficult to achieve yield gains in the Canada Western Red Spring wheat class (Wang et al., 2002). The positive relationship between grain yield and days to maturity poses another challenge to wheat breeders in northern growing regions.

The goal of this thesis was to improve our understanding of the genetic control of flowering/maturity time in Canadian spring wheat. The specific objectives were 1) to investigate the importance of vernalization response and earliness *per se* genes in determining flowering/maturity time; 2) to study the inheritance of flowering/maturity time; 3) to identify specific *Vrn* gene combinations that may offer advantages in western Canadian growing conditions, and; 4) to study the genetic relationship of maturity time with grain yield and grain protein content. Results presented in the thesis are summarized and discussed in this chapter.

7.2 Genetic control of flowering/maturity time in Canadian spring wheat

Five spring wheat cultivars, spanning the range in maturity of western Canadian spring wheat, were studied to investigate the genetic control of flowering/maturity time. The five spring wheat cultivars differed in photoperiod and vernalization response, and earliness *per se* when tested in a controlled environment. The two late cultivars (AC Taber and AC Foremost) responded to a vernalization treatment by flowering 14 and 11 days earlier than when not vernalized, respectively. This suggests that these cultivars carry vernalization sensitive spring habit *Vrn* alleles. The two early maturing cultivars (AC Intrepid and Cutler), and the medium maturing cultivar (AC Barrie) did not respond to vernalization, indicating that their spring habit *Vrn* alleles are different than those of 'AC Taber' and 'AC Foremost'. 'AC Barrie' did not flower within 100 days after transplanting when grown in a short (10 hrs) photoperiod, thereby exhibiting photoperiod sensitivity. When vernalized and grown in long day conditions, 'AC Barrie' also differed in earliness *per se* from 'AC Taber', 'AC Foremost', 'Cutler' and 'AC Intrepid'. These results suggested that the relative lateness of 'AC Taber' and 'AC Foremost' in the field is due to their vernalization response which is most likely not fulfilled during the growing season, and that 'AC Barrie' has a longer basic vegetative phase (lateness *per se*) than the

other cultivars tested. Both 'AC Taber' and 'AC Foremost' have most probably inherited their vernalization responsiveness from their common parent 'HY320' (Cutforth et al., 1992).

Genetic analyses of the flowering/maturity time of the five cultivars were conducted in a controlled environment and in the field; on the F_1 and F_2 crosses obtained from a one-way diallel mating design. Flowering/maturity time was mainly controlled by additive gene action. Non-additive effects of smaller magnitudes were also observed for flowering/maturity time in both a controlled environment and in the field. The involvement of additive effects in the inheritance of heading/maturity time in spring wheat was previously reported by Bhatt (1972), Klaimi & Qualset (1974), Nanda et al. (1981), Sheikh et al. (2000) and Singh et al. (2003). Non-additive effects were also found important in controlling heading/maturity time of spring wheat (Klaimi & Qualset, 1974; Nanda et al., 1981). Narrow-sense heritability for flowering/maturity time was high (>60%) when not vernalized in both a controlled environment and in the field.

Based on the segregation behaviour in the F_2, the five parents fell into two distinct groups, with 'AC Taber' and 'AC Foremost' being one and 'AC Barrie', 'Cutler' and 'AC Intrepid' being the other group. Crosses between groups produced winter type segregants in the F_2 generation with no exceptions. Crosses within groups did produce some transgressively late segregants but no winter types. The occurrence of winter types in the F_2 of crosses between groups indicated that 'AC Taber' and 'AC Foremost' carry different spring habit alleles at the major *Vrn* loci, than the other three cultivars. The segregation patterns in the crosses between groups fitted the expected ratio of 15:1 for two gene models in both years (2004-05). Further molecular analyses revealed that 'AC Foremost' and 'AC Taber', genotypes of the Canadian Prairie Spring Wheat class, carried the dominant *Vrn-B1* and the recessive *vrn-A1* alleles; 'AC Barrie', 'Cutler' and 'AC Intrepid' carried the dominant *Vrn-A1* and recessive *vrn-B1* alleles.

7.3 Vernalization response genes in Canadian spring wheat

Molecular characterization of 42 Canadian spring wheat cultivars released from 1885 to 2004 revealed the presence of spring habit allele of *Vrn-A1* in 83% of the cultivars/lines. The spring habit allele of *Vrn-B1* was found in 50% of the cultivars/lines. About 36% of the cultivars/lines carried spring habit alleles of *Vrn-A1* and *Vrn-B1* in combination. This combination of *Vrn-A1* and *Vrn-B1* alleles was more prevalent in cultivars/lines registered after 1996. Sixty seven percent of Canada Prairie Spring Wheat cultivars tested had *Vrn-B1* as their sole spring habit allele, which conferred lateness. All cultivars/lines carried the winter habit allele at *Vrn-D1*. The predominance of the spring habit allele *Vrn-A1* in Canadian spring wheat appears to be due to the vernalization insensitivity of *Vrn-A1*, which confers earliness under non-vernalizing growing conditions. Wheat breeders in western Canada have incorporated the *Vrn-A1* allele into spring wheats mainly by selecting for early maturing genotypes for a short growing season.

7.4 Effects of vernalization genes on earliness and related agronomic traits

This study was conducted to investigate the effects of *Vrn* genes and seeding time on flowering/maturity time, and related agronomic traits of spring wheat. Sixteen genotypes, including a set of reciprocal whole-chromosome substitution lines (carrying different *Vrn* genes) in the 'Cadet'/'Rescue' hard red spring wheat background, and eight western Canadian spring wheat cultivars of known *Vrn* genes, were grown over three seeding dates, two weeks apart (starting early May), at two locations in central Alberta, Canada during 2004-05. Seeding date altered flowering/maturity times, plant height, grain yield and grain protein, but not grain weight of all genotypes. Grain yield decreased with delayed seeding. Although significant genotype × seeding date interactions were observed within each year and location for flowering/maturity time, these interactions did not follow a pattern which could be ascribed to the vernalization response of the genotypes. With few exceptions, the relative flowering/maturity times (°C days) or ranks of the genotypes did not change from one seeding date to another. These results suggest that the vernalization response of spring-sown wheat is most likely not fulfilled in western Canadian growing conditions.

The genotype carrying spring habit alleles at *Vrn-A1*, *Vrn-B1* and *Vrn-D5* matured the earliest, had the highest grain protein content but lowest grain yield. Genotypes with spring habit alleles *Vrn-A1* and *Vrn-B1* were early maturing and relatively high yielding. Genotypes carrying only spring habit alleles of *Vrn-B1* or *Vrn-D5*, or a combination of *Vrn-D5* and *Vrn-A1* were late maturing and high yielding. The spring habit allele of *Vrn-A1* was not completely epistatic to *Vrn-B1* and *Vrn-D5* for flowering/maturity time. The spring habit allele of *Vrn-B1*, however, was epistatic to that of *Vrn-D5* for these traits. Shindo et al. (2003) also reported that the spring habit allele of *Vrn-B1* was epistatic to spring habit allele of *Vrn-D1*.

7.5 Genetic relationship of maturity with grain yield and protein in spring wheat

The objective of this study was to investigate the genetic relationship between maturity, grain yield and grain protein content in a population of 130 CIMMYT spring wheat lines selected during 1998 for their earliness at Ciudad Obregon, Sonora state, Mexico. Field trials were conducted at the Edmonton Research Station of the University of Alberta, Edmonton, AB Canada and at the Field Crop Development Centre, Lacombe, AB Canada, during 2003 and 2004. Time to maturity was positively correlated with grain yield (0.69), harvest index (0.45), grain fill duration (0.70), grain fill rate (0.47) and grains spike^{-1} (0.68), while negatively correlated with grain protein (-0.87) and number of spikes (-0.44). Grain protein content exhibited negative genetic correlation with grain fill duration (-0.78), grain fill rate (-0.49), grain yield (-0.93) and harvest index (-0.71). Grain yield exhibited positive genetic correlation with grain fill rate (0.78), grain fill duration (0.49) and harvest index (0.55). Broad-sense heritabilities were low (<0.40) for rate and duration of grain fill, grain protein content, spike m^{-2}, grains spike^{-1}, harvest index, and grain yield, and medium to high (>0.40) for grain weight, days to anthesis and maturity, and plant height.

Despite the negative association between grain yield and grain protein content, some lines departed from the general interrelationships of maturity, yield and protein content. Lines with average grain yield but significantly higher grain protein than the

population mean were identified. Similarly, lines with average maturity and significantly higher grain yield than the population mean were also identified. The existence of such lines, and the considerable genetic variability exhibited for maturity, grain yield and grain protein content, suggests the possibility of simultaneously improving the three traits in global wheat germplasm.

7.6 General discussion

Earliness in wheat is an extremely complex trait and is controlled by three genetic systems, i.e., photoperiod response, vernalization requirement and earliness *per se*. The role of vernalization response and earliness *per se* genes on the flowering/maturity time of Canadian spring wheat has been described in this thesis.

Knowledge of the specific genes governing the range in maturity of Canadian spring wheat is useful for modifying flowering and maturity times of wheat grown in these regions. This thesis has demonstrated that both vernalization response and earliness *per se* genes play important roles in the flowering/maturity time of Canadian spring wheat. The lateness of the Canadian Prairie Spring Wheat class cultivars was found to be mainly due to their vernalization sensitivity. Most of the cultivars/lines of Canadian Western Red Spring Wheat class were found having the vernalization insensitive *Vrn-A1* allele, indicating that earliness *per se* genes play an important role in the genetic variation in the maturity time of the cultivars in this class.

Knowledge of the nature of gene action controlling flowering and maturity times of spring wheat is beneficial in formulating breeding strategies to modify these traits according to the needs of a given growing environment. The overall genetic effect of vernalization response and earliness *per se* genes on time to flowering/maturity of Canadian spring wheat was mainly additive. The preponderance of additive genetic effects suggests that selection for early flowering/maturity in early generations will result in genetic improvement towards earliness. Initial selection against major vernalization response genes (by selecting for early genotypes in the field) could result in early flowering and maturity at the beginning of a selection program. Following the

elimination of vernalization response, selection for earliness *per se* genes could fine-tune flowering and maturity time for selected environments. The flowering/maturity times of Canadian spring wheat may also be modified by incorporating two or three spring habit alleles at the major *Vrn* loci.

Results of this thesis identified combinations of *Vrn* genes that may offer advantage in northern spring wheat growing regions. Genotypes carrying spring habit alleles at three *Vrn* loci may provide extreme earliness in regions with relatively short growing season. Although grain yields of such genotypes may be comparatively lower than their late counterparts, these genotypes may be less prone to damage by early fall frost. The presence of spring habit alleles at *Vrn-A1* and *Vrn-B1* appears to be a preferred *Vrn* genotype in high northern latitudes. This genotype combines both earliness and acceptable grain yields. Some of the elite Canadian spring wheat cultivars (Park, CDC Go, McKenzie, and Superb) possess this *Vrn* combination. This suggests that in the northern wheat growing regions, breeding preference may be given to genotypes carrying three spring habit alleles, or to those having spring habit alleles of *Vrn-A1* and *Vrn-B1*. Genotypes carrying the vernalization sensitive spring habit *Vrn* alleles (*Vrn-B1*, *Vrn-D1*, and *Vrn-D5*) either singly or in combination tend to mature comparatively later than those with *Vrn-A1*. The former genotypes may have greater grains spike^{-1} and grain weights due to their longer vegetative and grain-fill period, and may yield well in regions with longer growing seasons. Such genotypes must be planted as early in the growing season as possible to avoid yield declines, which we observed in Chapter 5 of this thesis.

The spring habit allele *Vrn-D1* was not found in any genotype examined in this thesis, suggesting that it has not been employed in spring wheat breeding programs in western Canada. However, a recent study concluded that *Vrn-D1* had the highest frequency among the major *Vrn* genes in the globally important CIMMYT wheat cultivars, with the semi-dwarf Mexican variety 'Sonora 64' being one of the varieties responsible for the wide distribution of the *Vrn-D1* gene (Van Beem et al., 2005). It may be interesting, therefore, to incorporate different combinations of dominant *Vrn* alleles, including those with dominant *Vrn-D1*, in spring wheat breeding programs in western

Canada. Molecular markers are now available for the major vernalization genes (*Vrn-A1*, *Vrn-B1* and *Vrn-D1*) in wheat (Yan et al., 2004; Fu et al., 2005), and they may now be directly selected using marker-assisted selection. This may aid in the selection of early maturing spring wheat cultivars with higher grain yield potential.

Identification and incorporation of earliness *per se* genes in Canadian spring wheat cultivars will further aid in modifying flowering time, especially if breeders wish to employ the vernalization-sensitive dominant *Vrn* alleles in their breeding programs. Preliminary results from an ongoing study aimed at mapping earliness *per se* QTL in Canadian spring wheat have revealed linkage between five microsatellite loci and QTL affecting earliness *per se*. The QTL identified appear to explain a fairly large amount of phenotypic variation in flowering time and have not been previously reported. The genomic regions are being targeted with more microsatellite markers to identify markers closest to the QTL. These QTL are likely to play an important role in modifying flowering time of spring wheat grown in Canada and elsewhere.

Early maturity, grain yield and grain protein content are generally negatively correlated and, therefore, difficult to combine in a single genotype. In Chapter 6 of this thesis, genotypes were identified that did not follow this general interrelationship. The departure of these genotypes was generally associated with shorter grain fill duration and higher grain fill rate. This suggests the possibility of developing early maturing spring wheat without negatively affecting grain yield and grain protein content, through selection for shorter grain fill duration and higher grain fill rate. However, a point of concern is the low heritabilities of these two traits, implying that genetic gain in these traits would require multi-environment testing. The issue of low heritability of grain fill duration may be solved by indirect selection for shorter grain fill duration through delayed anthesis and early maturity, both exhibiting relatively high heritabilities.

7.7 Conclusions

The following provides a summary of the conclusions drawn from this thesis:

- Vernalization response and earliness *per se* genes play important roles in the flowering/maturity time of Canadian spring wheat.
- The overall genetic effects of vernalization response and earliness *per se* genes are additive in nature.
- The spring growth habit of western Canadian spring wheat is mainly determined by the spring habit alleles of *Vrn-A1* and/or *Vrn-B1*.
- The spring habit allele of *Vrn-D1* has not been employed in spring wheat breeding programs in western Canada.
- The vernalization requirements of sensitive spring wheat cultivars are most likely not fulfilled under western Canadian growing conditions in most years.
- Incorporating different combinations of spring habit *Vrn* alleles may help in developing early maturing spring wheat cultivars with relatively high grain yields.
- Early generation selection for *Vrn* genes followed by selection for earliness *per se* and grain yield in later generations, would be an appropriate breeding strategy in wheat breeding programs in western Canada.
- Wheat genotypes exist that do not follow the general interrelationship of maturity, grain yield and grain protein content.
- Selection for longer pre-anthesis and shorter grain fill periods may help circumvent the negative association between grain yield and grain protein content.
- Selection for shorter grain fill periods and higher grain fill rate may be a useful strategy for developing early maturing cultivars with acceptable grain yields.

7.8 Contribution to Knowledge of Earliness in Wheat

Despite the existence of considerable genetic variation for maturity time in western Canadian spring wheat, the genetic basis of these differences is poorly understood. The contribution of this thesis to the knowledge of earliness in wheat is discussed in the following paragraphs.

There have been very few reports on the vernalization responses of Canadian spring wheat (Major & Wheelan, 1985; Roberts & Larson, 1985; Jedel et al., 1986). Similarly, there has been only one report, to the best of my knowledge, on the inheritance of vernalization response genes (Jedel, 1994). Experiment 1 in Chapter 2 of this thesis is one of the few reports on the genetic control of flowering/maturity time of Canadian spring wheat. Experiment 2 in Chapter 2 is, to the best of my knowledge, the first ever report on the diallel analysis of vernalization response and earliness *per se* in spring wheat. Through these studies, I have demonstrated not only the effects of vernalization response and earliness *per se* genes on flowering/maturity time, but also the gene action involved in the inheritance of the traits. These results have advanced the knowledge of earliness in wheat.

Chapter 3 of this thesis is, to the best of knowledge, the first report on the genetic analysis of flowering and maturity time in Canadian spring wheat. This study has demonstrated the importance of vernalization response and earliness *per se* genes and the preponderance of additive genetic effects in the control of flowering/maturity time in Canadian spring wheat which is advancement of knowledge in this domain.

Chapter 4 of this thesis is the first report, to the best of my knowledge, on the molecular characterization of *Vrn* genes in Canadian spring wheat. The *Vrn* genes of very few Canadian spring wheat cultivars (Cadet, Marquis, Reward and Thatcher) have been characterized before (Yan et al., 2004; Fu et al., 2005). Results of this study could have a significant impact on future spring wheat breeding in western Canada.

There have been few reports on the effects of *Vrn* genes on important agronomic traits in spring wheat (Stelmakh, 1993; 1998). Chapter 5 of this thesis is the first report on the effects of *Vrn* genes on related agronomic traits in Canadian spring wheat. These results could serve as guidelines for wheat breeders in formulating breeding strategies.

The interrelationship of maturity time, grain yield and grain protein content in wheat has been the subject of debate in the literature (e.g. Nass & Reiser, 1975; Stewart & Dwyer, 1990; Sharma, 1994). The association between these traits has been found

variable across environments and populations. The examination of these associations in Chapter 6 provides more information for this debate. Moreover, genotypes departing from the general interrelationship of these traits have been identified that may serve as useful genetic material for further study.

This thesis, as a whole, constitutes an "advancement of knowledge in the domains in which the research was conducted". The importance and mode of action of *Vrn* and *eps* genes in the genetic control of flowering/maturity time of Canadian spring wheat has been determined; *Vrn* genes of Canadian spring wheat and specific *Vrn* gene combinations that may offer advantage in western Canada were identified; strategies for breeding early maturing spring wheat with relatively high grain yield were recommended. This research may help in the development of early maturing spring wheat cultivars with acceptable grain yield in western Canada.

7.9 References

Bhatt, G.M. 1972. Inheritance of heading date, plant height, and kernel weight in two spring wheat crosses. Crop Sci. 12: 95-98.

Cutforth, H. W., Y.W. Jame, and P.G. Jefferson. 1992. Effect of temperature, vernalization and water stress on phyllochron and final main-stem leaf number of HY320 and Neepawa spring wheats. Can. J. Plant Sci. 72: 1141-1151.

Fu, D., P. Szűcs, L. Yan, M. Helguera, J.S. Skinner, J. von Zitzewitz, P.M. Hays, and J. Dubcovsky. 2005. Large deletions within the first intron in *VRN-1* are associated with spring growth habit in barley and wheat. Mol. Genet. Genomics, 273: 54-65.

Gororo, N.N., R.G. Flood, R.F. Eastwood, and H.A. Eagles. 2001. Photoperiod and vernalization responses in *T. turgidum* × *T. tauschii* synthetic hexaploid wheats. Ann. Bot. 88: 947-952.

Jedel, P.E. 1994. Inheritance of vernalization response in three populations of spring wheat. Can. J. Plant Sci. 74: 753-757.

Jedel, P.E., L.E. Evans, and R. Scarth. 1986. Vernalization responses of a selected group of spring wheats (*Triticum aestivum* L.) cultivars. Can. J. Plant Sci. 66: 1-9.

Kato, K., and H. Yamagata. 1988. Method for evaluation of chilling requirement and narrow-sense earliness of wheat cultivars. Japan. J. Breed. 38: 172-186.

Klaimi, Y.Y., and C.O. Qualset. 1974. Genetics of heading time in wheat (*Triticum aestivum* L.). II. The inheritance of vernalization response. Genetics, 76: 119-134.

Major, D.J., and E.D.P. Whelan. 1985. Vernalization and photoperiod response characteristics of a reciprocal substitution series of rescue and Cadet hard red spring wheat. Can. J. Plant Sci. 65: 33-39.

Nanda, G.S., G.N. Hazarika, and K.S. Gill. 1981. Inheritance of heading date, plant height, ear length and spikelets per spike in an intervarietal cross of wheat. Theor. Appl. Genet. 60: 167-171.

Nass, H.G., and B. Reiser. 1975. Grain filling period and grain yield relationships in spring wheat. Can. J. Plant Sci. 55: 673-678.

Poehlman, J.M., and D.A. Sleper. 1995. Breeding Field Crops. Iowa State University Press, Ames, Iowa, USA, p-151.

Roberts, D.W.A., and R.I. Larson. 1985. Vernalization and photoperiod responses of selected chromosome substitution lines derived from 'Rescue', 'Cadet' and 'Cypress' wheats. Can. J. Genet. Cytol. 27: 586-591.

Sharma, R.C. 1994. Early generation selection for grain-filling period in wheat. Crop Sci. 34: 945-948.

Sheikh, S., I. Singh, and J. Singh. 2000. Inheritance of some quantitative traits in bread wheat (*Triticum aestivum* L. em Thell.). Ann. Agric. Res. 21: 51-54.

Shindo, C., H. Tsujimoto, and T. Sasakuma. 2003. Segregation analysis of heading traits in hexaploid wheat utilizing recombinant inbred lines. Heredity, 90: 56-63.

Stelmakh, A.F. 1993. Genetic effect of *Vrn* genes on heading date and agronomic traits in bread wheat. Euphytica, 65: 53-60.

Stelmakh, A.F. 1998. Genetic systems regulating flowering response in wheat. Euphytica, 100: 359-369.

Stewart, D.W., and L.M. Dwyer. 1990. Yields and protein trends of spring wheat (*Triticum aestivum* L.) on the Canadian prairies, 1961-1982. Can. J. Plant Sci. 70: 33-44.

Van Beem, J., V. Mohler, R. Lukman, M. van Ginkel, M. William, J. Crossa, and A.J. Worland. 2005. Analysis of genetic factors influencing the developmental rate of globally important CIMMYT wheat cultivars. Crop Sci. 45: 2113-2119.

Wang, H., T.N. McCaig, R.M. DePauw, F.R. Clarke, and J.M. Clarke. 2002. Physiological characteristics of recent Canada western red spring wheat cultivars: Yield components and dry matter production. Can. J. Plant Sci. 82: 299-306.

Yan, L., M. Helguera, K. Kato, S. Fukuyama, J. Sherman, and J. Dubcovsky. 2004. Allelic variation at the *VRN-1* promoter region in polyploidy wheat. Theor. Appl. Genet. 109: 1677-1686.

Appendices

Appendix 1. Pedigrees of 130 CIMMYT wheat lines used to study the genetic relationship between maturity, grain yield and grain protein content.

Line	Parentage
CIMMYT001	URES/BOW//OPATA
CIMMYT002	TOBARITO M 97
CIMMYT003	PJN/BOW//OPATA
CIMMYT004	KAUZ/CMH77.308//BAU
CIMMYT009	HANH/2*WEAVER
CIMMYT010	INIFAP M 97
CIMMYT016	CLC89/RABE
CIMMYT017	OPATA/RAYON/KAUZ
CIMMYT018	OPATA/RAYON/KAUZ
CIMMYT019	SKAUZ*2/SRMA
CIMMYT020	SKAUZ/PARUS//PARUS
CIMMYT021	HE1/3*CNO79//2*SERI/3/ATILLA
CIMMYT024	BOW/CROW//BUC/PVN/3/2*VEE#10
CIMMYT025	KAUZ/GEN
CIMMYT027	BOW/CROW//BUC/PVN/3/VEE#10
CIMMYT029	CS/TH.SC//3*PVN/3/MIRLO/BUC
CIMMYT032	BAKHTAWAR 94
CIMMYT042	PRL/SARA/TSI/VEE#5
CIMMYT043	VEE/PJN//2*TUI
CIMMYT044	VEE/PJN//2*TUI
CIMMYT048	BOW/CROW//BUC/PVN/3/2*VEE#10
CIMMYT056	KVZ/BJY
CIMMYT059	HPO//PJ62/COQ
CIMMYT061	VEE/PJN//2*TUI
CIMMYT063	WL6736/2*WEAVER
CIMMYT064	CMH77.308
CIMMYT077	SERI M 82
CIMMYT078	RAYON F 89
CIMMYT089	VEE#5/SARA
CIMMYT090	PASTOR
CIMMYT091	ALD/COC//URES
CIMMYT095	GAA/PRL
CIMMYT096	NG8319//SHA4/LIRA
CIMMYT097	SHA3/SERI//SHA4/LIRA
CIMMYT104	TUI
CIMMYT105	TRAP#1/BOWTIBA 63E//IN*2/KR/5/TOB/CNO67/4/NAR59/3/FN/K58//
CIMMYT107	ARANDAS F 90
CIMMYT108	BATAN F 96
CIMMYT110	PASTOR
CIMMYT115	ALDPVN//BOW/3/LIRA
CIMMYT117	ODK16/PDGA//AU/JTS179/3/NAC/4/OPATA/5/CNO79/PRL
CIMMYT118	JUP/ZP//COC/3/PVN/4/TNMU/5/TNMU
CIMMYT119	NANJING 8508/3/CHUM18//JUPBJY
CIMMYT120	SHA4/3/2*CHUM18//JUP/BJY

Appendix 1 (continued)

Line	Parentage
CIMMYT124	CHUM18/SERI
CIMMYT126	CETTIA
CIMMYT127	CATBIRD
CIMMYT130	TSI/VEE#5//BOW
CIMMYT131	PRL/VEE#6//VEE/MYNA
CIMMYT135	ALD/COC//URES
CIMMYT136	SHA8//PRL/VEE#6
CIMMYT137	BR23/PF869107
CIMMYT143	LIRA/TAN
CIMMYT151	CEP8927
CIMMYT153	BR. 16
CIMMYT154	IAS58/4/KAL/BB//CJ71/3/ALD/5/VEE#7
CIMMYT158	KAUZ*2//K134(60)/VEE
CIMMYT159	PJN/BOW//OPATA
CIMMYT160	VEE/PJN//2*TUI
CIMMYT161	FLYCATCHER
CIMMYT162	SITTA
CIMMYT167	GEN*2//BUC/FLK
CIMMYT169	BJY/COC//PRL/BOW
CIMMYT172	HAAS3621-2/3/F60314.76/MRL//CNO79
CIMMYT174	KACINO
CIMMYT175	FLYCATCHER
CIMMYT182	SHA3/SERI//SHA4/LIRA
CIMMYT185	CHIR6/3/LIRA/FFN//VEE#5/4/MAYOOR
CIMMYT186	CS/TH.CU//GLEN/3/GEN/4/MYNA/VUL/5/FANG60/6/CHIR6
CIMMYT190	TAM200/TUI
CIMMYT191	MAYOOR/SABUF//MAYOOR
CIMMYT193	CHIRYA.3
CIMMYT194	CS/TH.CU//GLEN/3/ALD/PVN/4/CS/LE.RE//2*CS/3/CNO79
CIMMYT199	BCN//DOY/AE.SQUARROSA (447)
CIMMYT205	HXL8088
CIMMYT214	PASTOR
CIMMYT217	CHEN/AEGILOPS SQUARROSA (TAUS)//BCN
CIMMYT224	CROC_1/AE.SQUARROSA (205)//KAUZ
CIMMYT234	PASTOR
CIMMYT248	BOW/PRL//BUC/3/LUAN
CIMMYT249	BOW/PRL//BUC/3/LUAN
CIMMYT250	RABE/PARUS//PARUS
CIMMYT251	"F3EHSVHB/PI"
CIMMYT256	MAYA/MON//CMH74A.592/3/2*GIZA157
CIMMYT259	WEAVER/ROBLIN
CIMMYT263	CLC89/RABE
CIMMYT264	TAM200/TURACO
CIMMYT267	PVN//CAR422/ANA/5/BOW/CROW//BUC/PVN/3/YR/4/TRAP#1
CIMMYT268	RL6043/4*NAC

Appendix 1 (continued).

Line	Parentage
CIMMYT272	"ONJPGLU3 352"
CIMMYT274	CMH73A.497/2*CNO79//CMH76.173/CNO79
	CMH81.38/2*KAUZCAL/NH//H567.71/3/2*NING
CIMMYT276	7840/CMH83.2277/5/BOW/2*NING 7840/4/CMH83.227
CIMMYT278	CAL/NH//H567.71/3/SERI/4/CAL/NH/H567.71/5/2*KAUZ/6/BAV92
CIMMYT279	CAL/NH//H567.71/3/SERI/4/CAL/NH/H567.71/5/2*KAUZ/6/BAV92
CIMMYT280	RABE/6/WRM/4/FN/3*TH//K58/2*N3/3
	AUS-869/5/PEL72380/ATR71/7/2*RABE/8/IRENARABE/6/WRM/4/FN/3*TH//
CIMMYT283	K58/2*N3/3
CIMMYT285	BAU/SERI
CIMMYT288	ALPHA/ADAM TAS//SST825
CIMMYT289	SW89.2089/3/HE1/3*CNO79//2*SERI
CIMMYT291	ALDAN/IAS58//SHA7
	MYNA/VUL//JUN/3/FRTL/4/TRAP#1/BOWCI14227/TRM//MAD/3/FAN1/4/NANJI
CIMMYT293	NG 82149
CIMMYT294	LIRA/5/LIRA/FFN//VEE#5
CIMMYT295	LIRA/5/LIRA/FFN//VEE#5
CIMMYT297	LIRA/5/LIRA/FFN//VEE#5CI14227/TRM//MAD/3/FAN1/4/NANJING 82149
CIMMYT300	BSP95.6
CIMMYT304	Not available
CIMMYT311	"KAUZ/AA//KAUZ"
CIMMYT312	"NG8319//SHA4/LIRA"
CIMMYT315	ZACATECAS VT74
CIMMYT322	STAR/KAUZ//STAR
CIMMYT332	"PYT/VAR 216
CIMMYT336	90B57/3/HE1/3*CNO79//2*SERI
CIMMYT337	CHUM 18/KAUZ
CIMMYT339	F60314.76/MRL//CNO79/3/CRDN/4/WEAVER
CIMMYT347	PASTOR/3/VEE#5//DOVE/BUCCHEN/AEGILOPS SQUARROSA
CIMMYT350	VORONA//CMH82A.1294/2*KAUZ
CIMMYT354	BORL95/3/HD2206/HORK/BUC/BUL/4/HE1/3*CNO79//2*SERI
CIMMYT357	THB/KEA/3/2*PEG//MRL/BUC
CIMMYT367	MIMUS/BIA_1//RAYONV763.2312/V879.C7.11.11.11(36)/3/HE1/3*CNO79//
	2*SERI/4/HE1/3*CNO79//2*SERIV763.2312/V879.C8.11.11.11(36)/3/HE1/3*CNO79
CIMMYT368	//
CIMMYT370	2*SERI/4/HE1/3*CNO79//2*SERI
CIMMYT371	V763.2312/V879.C811.11.11(36)//STAR/3/STAR
CIMMYT372	TUI (CIMMYT,ACC 13725)
CIMMYT374	CBSME1FE 230 ACC17447
CIMMYT377	CBSME1FE 142 ACC19770
CIMMYT378	CBSME1FE 232 ACC19823
CIMMYT380	CBSME1FE 294 ACC20125

Appendix 2. Least squares means for 7 traits of 130 CIMMYT wheat lines/cultivars and 10 Canadian spring wheat cultivars grown at two locations in central Alberta, Canada during 2003-04.

Genotype	Anthesis (days)	Maturity (days)	GFD (days)	GFR (kg ha^{-1} day^{-1})	Grain weight (mg)	Grain yield (t ha^{-1})	Grain protein (%)
CHIL408	67	107	44	108	39.3	3.98	12.0
CIM001	69	108	42	138	47.7	4.57	12.3
CIM002	67	103	39	153	36.9	4.80	12.1
CIM003	66	108	44	117	40.6	4.24	12.3
CIM004	70	112	43	143	42.8	4.80	11.0
CIM009	67	105	42	135	42.4	4.54	12.4
CIM010	67	107	44	132	42.8	4.73	12.4
CIM016	66	103	41	126	36.3	4.06	12.6
CIM017	69	107	40	161	44.2	4.89	12.2
CIM018	69	108	41	143	44.7	4.74	12.5
CIM019	67	106	41	119	37.8	4.21	12.4
CIM020	68	109	43	145	40.1	5.01	11.4
CIM021	69	108	42	142	45.0	4.67	11.9
CIM024	65	100	40	129	41.7	4.10	12.4
CIM025	68	107	43	140	41.0	4.47	12.7
CIM027	65	104	42	121	40.5	4.01	12.6
CIM029	66	107	44	101	40.4	4.25	11.6
CIM032	67	106	42	135	36.8	4.75	12.5
CIM042	68	107	41	122	38.2	4.16	12.2
CIM043	69	110	43	136	40.0	4.75	11.7
CIM044	67	107	42	131	41.3	4.41	12.6
CIM048	65	105	44	113	38.3	4.00	12.5
CIM056	66	106	43	126	38.8	4.51	12.2
CIM059	65	102	41	139	41.9	4.49	12.6
CIM061	68	107	41	151	42.3	4.84	12.8
CIM063	66	105	43	120	42.6	3.97	12.5
CIM064	66	107	44	134	36.5	4.63	11.8
CIM077	68	107	42	147	39.4	5.20	11.9
CIM078	68	106	41	128	36.1	4.42	12.1
CIM089	68	107	42	129	44.3	4.26	13.1
CIM090	69	110	43	152	40.9	5.36	10.8
CIM091	67	105	41	116	38.9	3.91	13.8
CIM095	67	106	42	128	37.6	4.34	12.4
CIM096	66	102	39	138	41.6	4.29	13.4
CIM097	67	104	40	132	42.2	4.24	12.6
CIM104	68	106	40	144	37.0	4.30	13.3

Appendix 2 (continued).

Genotype	Anthesis (days)	Maturity (days)	GFD (days)	GFR (kg ha^{-1} day^{-1})	Grain weight (mg)	Grain yield (t ha^{-1})	Grain protein (%)
CIM105	69	109	42	153	39.9	5.10	11.3
CIM107	68	106	40	145	38.0	4.75	12.5
CIM108	67	107	44	118	45.2	4.25	12.1
CIM110	69	111	45	147	42.4	5.23	10.8
CIM115	70	111	43	136	40.2	4.79	11.6
CIM117	67	105	42	133	45.6	4.81	12.6
CIM118	67	104	41	137	40.0	4.48	12.2
CIM119	68	106	40	152	40.3	4.66	12.1
CIM120	69	109	43	139	41.4	4.63	11.4
CIM124	69	108	41	139	42.9	4.55	11.4
CIM126	66	107	44	121	38.6	4.28	12.0
CIM127	70	108	40	149	37.7	4.47	11.7
CIM130	70	111	43	139	38.3	4.80	11.6
CIM131	67	109	44	117	43.7	4.29	13.7
CIM135	67	105	40	109	41.1	3.50	14.1
CIM136	67	105	39	117	39.3	3.61	12.9
CIM137	67	105	41	108	35.4	3.55	13.3
CIM143	67	107	44	117	43.7	4.04	12.7
CIM151	69	107	41	162	39.7	5.05	11.3
CIM153	67	105	41	139	43.6	4.37	12.8
CIM154	66	104	40	146	48.5	4.61	12.4
CIM158	65	102	41	128	43.4	4.34	13.2
CIM159	68	107	42	119	44.3	4.15	12.6
CIM160	68	108	43	125	41.7	4.36	12.9
CIM161	66	106	44	115	42.3	4.06	12.2
CIM162	68	109	44	115	37.8	4.02	12.6
CIM167	70	112	44	117	39.0	4.24	12.1
CIM169	66	107	44	127	43.6	4.63	11.8
CIM172	68	113	48	115	36.9	4.50	12.1
CIM174	64	105	44	137	41.8	4.57	12.7
CIM175	65	102	41	107	47.2	3.59	12.9
CIM182	66	105	41	130	41.6	4.17	13.3
CIM185	66	106	43	124	38.4	4.07	13.3
CIM186	68	108	42	121	43.6	4.05	12.7
CIM190	66	109	46	116	39.4	4.46	12.0
CIM191	69	108	42	132	35.3	4.28	12.9
CIM193	68	108	42	143	42.7	4.74	11.8
CIM194	69	109	43	139	43.7	4.56	11.5

Appendix 2 (continued).

Genotype	Anthesis (days)	Maturity (days)	GFD (days)	GFR (kg ha^{-1} day^{-1})	Grain weight (mg)	Grain yield (t ha^{-1})	Grain protein (%)
CIM199	64	101	40	103	32.3	3.24	13.4
CIM205	70	109	41	140	38.7	4.55	12.7
CIM214	70	110	42	146	42.5	4.92	11.7
CIM217	65	106	43	137	45.7	4.89	11.6
CIM224	68	107	41	117	42.0	3.73	13.8
CIM234	66	107	45	131	39.4	4.76	11.3
CIM248	68	105	39	157	45.1	4.51	13.7
CIM249	69	114	47	110	43.2	4.15	11.6
CIM250	63	95	37	114	41.9	3.50	14.0
CIM251	70	108	40	133	36.8	4.31	11.9
CIM256	65	104	43	113	39.0	3.81	12.6
CIM259	63	95	36	122	40.8	3.55	13.8
CIM263	65	103	40	125	36.3	4.29	11.6
CIM264	68	107	42	136	41.9	4.79	11.6
CIM267	69	107	40	127	37.1	4.38	12.0
CIM268	66	104	41	114	40.2	4.01	12.3
CIM272	66	108	45	122	42.9	4.50	11.6
CIM274	63	98	40	81	38.2	2.43	14.6
CIM276	68	101	37	148	39.4	4.06	14.7
CIM278	66	103	41	132	40.3	4.51	12.5
CIM279	68	107	43	140	38.5	4.91	10.9
CIM280	66	103	40	118	37.7	4.19	11.3
CIM283	65	105	44	120	40.1	4.15	11.8
CIM285	65	102	40	107	38.6	3.38	14.1
CIM287	65	103	43	94	42.0	3.41	12.9
CIM288	66	104	41	112	35.1	3.86	12.3
CIM289	66	105	42	114	37.5	3.99	12.4
CIM291	64	103	41	126	36.1	4.18	12.0
CIM293	67	100	34	146	40.1	4.08	12.8
CIM294	67	100	35	143	41.0	4.02	13.4
CIM295	66	99	35	144	39.3	3.88	13.7
CIM297	65	101	39	131	38.7	3.95	13.1
CIM300	65	106	45	101	40.7	3.57	11.9
CIM304	68	106	40	141	37.5	4.81	11.9
CIM311	68	107	42	121	39.0	4.45	11.6
CIM312	65	100	37	140	41.3	4.14	13.3
CIM315	65	102	41	133	45.0	4.35	12.7

Appendix 2 (continued).

Genotype	Anthesis (days)	Maturity (days)	GFD (days)	GFR (kg ha^{-1} day^{-1})	Grain weight (mg)	Grain yield (t ha^{-1})	Grain protein (%)
CIM322	64	105	44	110	39.0	4.12	13.1
CIM332	63	101	43	122	37.8	4.08	13.4
CIM336	68	104	38	139	44.2	4.34	12.5
CIM337	69	108	41	131	38.0	4.41	12.1
CIM339	68	104	39	109	42.0	3.31	14.1
CIM347	65	105	42	119	42.7	4.13	12.5
CIM350	66	107	45	115	40.1	4.37	12.6
CIM354	66	107	44	132	39.0	4.68	13.0
CIM357	69	112	46	126	44.0	4.82	11.4
CIM367	68	107	42	121	35.8	3.89	13.5
CIM368	69	109	43	135	39.8	4.70	12.0
CIM370	69	109	43	131	40.7	4.72	12.5
CIM371	67	109	44	101	46.6	3.75	12.6
CIM372	67	105	40	140	39.1	4.66	12.2
CIM374	69	110	43	145	42.3	5.13	11.1
CIM377	67	101	37	136	37.9	3.79	14.3
CIM378	69	110	44	134	43.3	4.74	11.6
CIM380	69	111	44	139	45.6	5.00	11.0
USA405	64	104	44	124	41.7	4.36	13.4
Barrie	66	105	40	124	38.6	4.14	12.9
Bluesky	66	103	39	144	45.5	4.41	12.7
Cutler	65	101	40	147	41.2	4.40	12.5
Foremost	67	109	44	128	42.1	4.77	11.5
Glenlea	68	107	41	131	45.9	4.37	12.4
Intrepid	64	98	37	131	39.8	4.10	12.9
Katepwa	66	99	36	134	37.2	4.14	12.8
Splendor	64	97	36	121	36.2	3.56	13.3
Taber	68	110	44	132	42.8	4.77	11.0
Vista	66	104	41	139	43.3	4.48	11.3

GFD= Grain fill duration
GFR= Grain fill rate.